ANALYSIS OF MICROBIAL CONTAMINATION AND SURFACE DEFECTS OF NEW AND USED NiTi ROTARY ENDODONTIC FILES- AN ATOMIC FORCE MICROSCOPY STUDY

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CERTIFICATE

This is to certify that this dissertation titled " **ANALYSIS OF MICROBIAL CONTAMINATION AND SURFACE DEFECTS OF NEW AND USED NiTi ROTARY ENDODONTIC FILES- AN ATOMIC FORCE MICROSCOPY STUDY** " is a bonafide record of work done by Dr.K.C.MADHU SUDHANAN under our guidance during the study period 2010 – 2013.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY,** in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY- CONSERVATIVE DENTISTRY AND ENDODONTICS, BRANCH IV.** It has not been submitted (partial or full) for the award of any other degree or diploma.

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ABSTRACT

Background: Manufacturing process leads to surface defects in endodontic files.

Aim:

The purpose of this study was to evaluate the microbial contamination and surface defects of new and used rotary endodontic files by atomic force microscope.

Materials and Methods:

Three groups of rotary endodontic file were used for the study. group1 (Protaper), group-2(M two), group-3(Wave one). Four new and used files from group-1, and group-2 were selected (GROUP-1-S1,S2,F1,F2), (GROUP-2- ISO size 10,15,20,25), one new and used file was selected from Group-3 (wave one) ISO size 25 primary file. All the files were analysed in 11 points along 6mm section of the tip. Quantitative measurement according to topographic deviation(RMS) was recorded. Data were analysed by paired sample *t* test.

Two rotary system of files were selected for identification of microbial contamination. Twenty four files from Group-1(Protaper), and twelve files from Group-2(M two) were used. The files were incubated in BHI broth, a positive cultures were obtained.

Results:

All the files showed surface irregularity irrespective of electro polishing. Root mean square value for finishing files were higher than shaping files. Protaper showed higher wear among the three experimental groups. Surface defects such as pits and microcracks were observed in new and used Protaper files. All the files in group-1 (Protaper) showed positive culture. *Bacillus subtilis* and *pseudomonas* were identified in the contaminated samples.

Conclusion:

Protaper showed surface defects such as pits and microcracks and underwent higher wear. RMS value of used file were higher than shaping files. Mtwo showed least wear among the three experimental groups. All the endodontic files should be sterilized before clinical use.

Key words:

Surface defects, micro cracks and pits, NiTi rotary instruments, Atomic force microscopy, infection control.

INTRODUCTION

Successful endodontic therapy requires combination of factors such as proper diagnosis, thorough cleaning and shaping of the pulpal space, followed by successful disinfection and three dimensional obturation of the pulpal space and a final restoration.[22]Mechanical preparation of the root canal were achieved with the use of hand instruments or rotary nickel titanium files.In most of the cases the root canal anatomy is more complicated with curvature in multiple position and planes which pose great challenge to the clinicians.[22]Recent advances in endodontic instrument design had made the clinician to achieve efficient and predictable cleaning and shaping.[37]

Traditionally cleaning and shaping of the root canal were carried out by stainless steel files. The main advantages of hand files include a good tactile perception of what the tip of the instrument is encountering. As a result ,the operator is able to differentiate between a solid impediment and a narrow canal,thus shows warning before the instrument separation, The main disadvantage of these file are their rigidity which is responsible for straightening and its consequences in the apical, middle third of the root canal. This results in transportation and canal aberrations including ledges, zippings, perforations and leave a significant portion of the canal wall un-instrumented.[22]

Most noteworthy advancement is the development of NiTi rotary instruments which was introduced by Walia et al in 1988 to endodontics. It has made root canal instrumentation safe , increased operator efficiency, minimizing time , maintaining the canal shape and centricity.[38]The NiTi instruments gained more popularity due to their super elastic property and they flex far more than stainless steel instruments before exceeding their elastic limits.[4]The hand held NiTi instruments are identical to that of engine driven rotary files . They are recommended for use in reaming or “modified balanced forces” motion.The mechanical stresses acting on a hand-operated instrument might differ from those on engine-driven instruments. Engine-driven instruments operate in continuous rotation and are mainly subjected to unidirectional torque.[40] Preliminary studies on the failure mode of hand NiTi instruments indicated that shear failure caused by torsional stresses was prevalent, whereas rotary instruments were more affected by fatigue failure. Moreover thesetwo instruments differs in working time, number of rotation, and operator efficiency.[40]

The major concern with the use of NiTi engine driven rotary instruments are unexpected seperation of instrument during clinical procedure with out warning. Removal of broken instrument may not be feasible all the time, which may jeopardize the endodontic clinical outcome.[24]

Fracture of NiTi instrument used in rotary motion occurs in two ways, fracture because of torsional fatigue,and flexural fatigue. Torsional fatigue occurs when the tip of the instrument gets locked in the canal whilst there is continuous rotation of shank, which leads to the separation of instrument. Flexural fatigue occurs due to metal fatigue, as a result of repeated tension compression cycles, caused by the rotation within the curved canal increasing cyclic fatigue.[24]

The most important factor which is involved in unexpected fracture is the inherent manufacturing defects of NiTi files. The machining of NiTi endodontic files is a complex procedure, generally resulting in surface flaws with high deformation such as metal strips, debris in addition to pits and blunt cutting edges.[24]

Surface flaws that arise from the superficial defects play an important role in instrument fracture illustrating the importance of surface quality.[1]Several studies have investigated the surface quality of NiTi instruments using SEM.[16] SEM resolves the structure down to nanometer scale. SEM gives a two-dimensional ‘photographic’ image of the samples, but cannot directly provide quantitative data regarding the topography.[2]

The ATOMIC FORCE MICROSCOPY is now a well established and documented technique to provide qualitative and quantitative information about the topography of a wide variety of materials. The ATOMIC FORCE MICROSCOPY technique reconstructs, in real time, the three-dimensional image of the sample topography and provides a three-dimensional image of the sample surface facilitating both interpretation and visualization.[17]

The atomic force microscopy (AFM) is a valuable research instrument for investigating the topography of endodontic files (Valois et al). By ATOMIC FORCE MICROSCOPY, it is possible to say that used instruments demonstrated greater deformations and wear on the surface (Inan et al) and that multiple cycles in the autoclave increased the depth of the irregularities on the surface of

NiTi rotary instruments (Valois et al). Topuz et al used ATOMIC FORCE MICROSCOPY to find out the surface deterioration of the endodontic files when they were immersed in 5.25% sodium hypochlorite.[2]

Infection control guidelines require the sterilization of instruments that come in contact with biological tissues. Sterilization is a process that destroys all the life forms to reduce the infection and cross infection. Since microorganisms have shown to be the major cause of endodontic disease process, sterilization of dental instruments becomes a mandatory step to maintain asepsis in endodontics.[33]

Endodontic file manufacturers do not disclose any claims over the product sterility, and they instruct the clinician to sterilize the new unused instrument before use. Elimination of microorganisms from the root canal contributes immensely to the success of endodontic therapy. Infection can occur due to entry of microbes to a sterile pulpal zone by a caries process or cross contamination by using unsterile instruments during endodontic therapy. These conditions affect the outcome of the endodontic treatment.Hence it is mandatory to use a sterile instrument. Only limited investigation has been carried out to determine the presence or absence of microbial contamination in unused new files received from the manufacturer.Todd P. Roth observed positive microbial cultures in new endodontic files. Close examination of unused endodontic files received from manufacturers has shown the presence of debris on their surfaces, including metallic spurs, grease, and even epithelial cells (Brady JM).[33]So it becomes a absolute necessity to investigate the sterile nature of endodontic instruments

AIM:

The aim of this invitro study was to

1. Evaluate the surface defects of new and used NiTi endodontic files using ATOMIC FORCE MICROSCOPE.
2. To determine the sterility of new endodontic files received from manufacturer.

OBJECTIVES:

The objective of this study was to

1. Evaluation of surface defects like pits and micro-cracks in Protaper, Mtwo, and wave one endodontic files after using them in twelve root canals.
2. Ascertain the sterility of new unused endodontic files.

REVIEW OF LITERATURE

Sattapan et al (2000)[30] evaluated the defects in rotary nickel titanium files after clinical use. Total of 378 files (Quantec series) which were discarded after the normal use were analyzed under stereomicroscope. The results showed more than 50% of the files showed visible defects, 21% were fractured and 28% showed defects apart from fracture. In fractured files group 55.7% were due to torsional fatigue and 44.3% of the files were due to flexural fatigue. He concluded that torsional fatigue occurs more frequently than flexural fatigue which is due to use of too much apical force during instrumentation.

S.A.Thompson et al (2000)[36]evaluated the property of nickel titanium alloy regarding the manufacturing, processing, characteristic feature. The nickel–titanium alloy is used in the manufacture of endodontic instruments in recent years. Nitinol alloys have greater strength and a lower modulus of elasticity compared with stainless steel alloys. The super-elastic behavior of Nitinol wires means that on unloading they return to their original shape following deformation. These properties are of interest in endodontology as they allow construction of root canal instruments that utilize these favourable characteristics to provide an advantage when preparing curved canals. This review aims to provide an overview of Nitinol alloys used in dentistry for its unique characteristics.

Tripi et al (2001)[35] evaluated the defects in rotary instruments before and after use. Before use the files were photographed in SEM. The instruments were used in 12 canals and they were cleaned and reexamined under SEM. The instruments showed the presence of debris, scraping and blunt cutting edge. He concluded that after clinical use the surface defects in GT rotary increased.

Klaus K daunt et al (2001)[17] reviewed the use of atomic force microscopy in biomaterials surface and interface. The use of atomic force microscopy in biomaterials science and engineering application has increased rapidly over the last few years. Beyond being merely a tool for measuring surface topography, AFM has made significant contributions to various biomaterials research areas dealing with the structure, properties dynamics and manipulation of biomaterials surface and interfaces. Selected examples presented include micro and nano structure and properties of biomaterials surface, molecular level interactions at biomaterial biomolecule interface, interface between biomaterials and mineralized tissue as well as advances of mineralized tissue research. In these areas AFM to be used as a versatile tool to study micro and nanostructure

Martin et al(2002)[19]evaluated the surface analysis on profile of instrument before use, after sterilization by 2 different method. The study included 3 groups. Group 1- 5 new profile instrument Group 2-5 new instrument (ISO 25),Group 3-5 new profile NiTi files(ISO 20)and analysed by SEM , Group-1 was autoclaved. Group was sterilized by dry heat. The instruments were used in six molar root canals. Results showed that along with the usual machining defects various carbon and sulphur which were found in the surface of the new instrument.

Timothy A Svec and Powers et al (2002)[34] evaluated the deterioration of rotary NiTi files under controlled condition. The study design included ISO size 20 of 0.04 taper rotary files, used in electric hand piece configured to rotate at 150 rpm with the load of 8N and a torsional moment failure was determined on a torsiometer. SEM of the file was taken before and after the use. The result showed torsional moment of the new and used NiTi was not affected by the use.

Alpati et al(2003)[2] observed the surface of new and used NiTi rotary files using SEM. Tip section of profile 0.04 taper and light speed 25mm long instruments were compared after 1, 3, 6 simulated extracted mandibular molar. Used Profile instrument showed flattening of characteristic material rollover and minor apparent wears at the edge of the flutes. Used Light speed instruments showed little change in the tip region. He concluded that deposits on the surface of the instruments were attributed to the manufacturing process which lead to the fracture of NiTi rotary files

Elio Berutti et al(2003)[6] investigated the comparative analysis of torsional and bending stress of nickel titanium rotary instruments by applying finite elemental analysis method to provide a numerical evaluation. The distributional of stress due to torsion and bending moments were compared in 2 experiments models. He concluded that Protaper model showed lower and better stress distribution than profile model.

Peters et al (2004)[23] evaluated the current challenges and concepts in the preparation of root canal system. He studied the factors influencing the shaping outcome. The factors included were preoperative root canal anatomy, instrument tip design, operator experience, rotational speed and instrument sequence. He concluded that even in the presence of risk factors the shaping outcome of nickel titanium instruments are mostly predictable

Fife et al(2004)[8] evaluated the cyclic fatigue of Protaper nickel titanium rotary instruments after multiple clinical use. 225 Protaper were divided into 3 groups. Group a-75 used as control, Group b-75 used in two molars, Group c- 75 used in 4 molar. The rotations to breakage and fractured tip length were recorded. The result indicated that no S1,S2,F1,F2,F3 instrument separated during intra-canal use . He concluded that prolonged reuse of NiTi rotary instruments strongly affects instrument fatigue.

Baumgartner et al(2004)[7] reviewed the microbiological and molecular methods used to study the microorganisms associated with endodontic infections. Over 500 species of bacteria have been cultivated from the oral cavity. Endodontic infections are poly-microbial with usually from 3–12 species cultivable from either infected root canals or peri-radicular abscesses. Molecular methods are able to detect and identify many additional species of bacteria associated with endodontic infections. Molecular methods provide precise identification of the microbes at the DNA level and detection of microbes that are not cultivable. Future research will detect and identify other as yet unknown species of bacteria, viruses, and fungi involved in endodontic infections. Which microorganisms produce virulence factors associated with serious endodontic infections will also be better defined using molecular methods. An increase in knowledge and understanding of the organisms associated with endodontic infections will improve our ability to clinically manage endodontic infections

Michael A Baumann (2004)[4] reviewed the challenges and options in Nickel titanium instrumentation. . The nickel–titanium alloy Nitinol is used in the manufacture of endodontic instruments in recent years. Nitinol alloys have greater strength and a lower modulus of elasticity compared with stainless steel alloys. The super-elastic behavior of Nitinol wires means that on unloading they return to their original shape following deformation. These properties are of interest in endodontology as they allow construction of root canal instruments that utilize these favourable characteristics to provide an advantage when preparing curved canals. This review aims to provide an overview of Nitinol alloys used in dentistry in order for its unique characteristics to be appreciated.

Maria Guiomar(2004)[20] evaluated the changes in fatigue resistance of nickel titanium rotary profile instruments after shaping 10 curved molar root canals were evaluated. Twenty five sets of file # 20, #25, #30 and taper of .04 and .06 were divided into two groups. He concluded that there is a stastistically significant decrease in number of cycles to failure than compared with new ones. The fracture point was same for all the files

Bahia and Buono et al(2005)[5] evaluated the changes in fatigue resistance of NiTi rotary profile instruments after clinical use in curved root canal system. The study comprises of 2 groups. GP-1 10 sets of new files GP 2-15 set of used files. Both the group tested in fatigue bench model. There was a significant decrease in number of cycles to failure in used files then to the new ones. He concluded that clinical use of profile instrument in curved canal reduces the cyclic fatigue.

Purificacion Varela Patino (2005)[26] evaluated the fracture rate of NiTi rotary instruments when following the manual glide path and using a stainless steel file before rotary instrumentation. The files were divided into three groups, 208 canals were selected GP1 – K3, GP 2- Profile, GP 3- Protaper and the apical part of the canals were enlarged with stainless steel files. He concluded that stainless steel file can be used in the apical 1/3 rd of the curved canals before introducing the files

Plotino et al(2006)[24]evaluated the cyclic fatigue of Mtwo NiTi rotary files after clinical use. The study design included 2 groups.GP-1 10 new instrument.GP-2 10 used instrument. Each instrument were used in 10 molar teeth. Cyclic fatigue testing was carried out in artificial canals with 5mm radius of curvature and 60 degree angle.Instrument were rotated until fracture and no of cycles to fracture were recorded. A statistical significant difference was noted between the 2 groups. He concluded that all the instruments had minimal instrument fatigue when discarded in controlled clinical use.

Ya Shen et al(2006)[40] evaluated the incidence and mode of instrument separation of two nickel titanium rotary file system according to clinical use. The study groups include a total of 166 Profile and 325 Protaper discarded from endodontic practice was analysed. Results showed the incidence of instrument separation were 7% for profile and14% for Protaper and propotion of unwinding defects was 5% in profile and 3% in Protaper. Flexural fatigue was the major reason for separation in two groups. He conclude that Protaper was more likely to separate with out warning, profile tended to exhibit unwinding of flutes more frequently.

Peter Parashos et al (2006)[25] reviewed the fracture of rotary NiTi instruments fracture and its consequence. He stated that the fracture of endodontic instruments is a procedural problem creating a major obstacle to normal routine therapy. Considerable research has been undertaken to understand the mechanisms of failure of NiTi alloy to minimize its occurrence. This has led to changes in instrument design, instrumentation protocols, and manufacturing methods. In addition, factors related to clinician experience, technique, and competence have been shown to be influential. From an assessment of the literature presented, we derive clinical recommendations concerning prevention and management of such complication.

Todd P Roth (2006)[33] conducted a study to test the sterility of new unused files received from the manufacturer. 15 types of hand and rotary files from the 5 manufacturer were selected and tested. Positive microbial cultures were obtained in this culture.He concluded that all the endodontic files should be sterilized before use

Herold K.S et al (2007)[13] evaluated the development of micro-fractures in the Endosequence nickel titanium rotary(NTR) files and Profile NTR using scanning electron microscopy(SEM) . He found that all Endosequence instruments developed micro-fractures by the seven canal evaluation, whereas the Profile instruments showed no micro-fractures at the 7-14 canal evaluation. It was also found that Endosequence files separated at a higher rate than Profile instruments

Wei et al(2007)[39] evaluated the modes of failure of Protaper NiTi rotary instruments after clinical use. Study design includes 100 fractured instruments. They were examined under stereo-microscope for plastic deformation along the cutting edge, near fracture site and Fracturographic and longitudinal examination were carried out in high power SEM. Results revealed that in 88 flexural and 12 torsional fatigue failure observed in the fractured file. Analysis of Fractured site by stereomicroscope revealed flexural fatigue with abrasion mark, cracks, micro-cracks, pitting.

Ya Shen et al(2007)[41] analysed of defects in Protaper. The study design included 401 hand protaper discarded from endodontic clinic over 6 months period. The failed instruments were examined on lateral and factrographic surface by SEM. Of 86 hand Protaper 28 were intact and 58 were fractured (36- shear, 22 –fatigue). Nearly 74% of the instrument fracture occurred at the apical 1/3rd of the canal. He concluded that most of the PHU instrument failed because of either shear of fatigue failure

Inan et al(2007)[16]evaluated the topography of new and used Protaper rotary nickel-titanium (NiTi) instruments by using atomic force microscope. Four new and four used size S1, S2, F1, and F2 instruments were selected for this study. New and used instruments were analyzed on 11 points along a 3-mm section at the tip of the instrument Mean root mean square values for used Protaper instruments were higher than the new ones, and the difference between them was statistically significant. The results of this study showed that used Protaper instruments demonstrated more surface deformation and wear.

Antonio bonaccorso et al(2007)[3] evaluated the surface properties of nickel titanium instruments emphasizing the importance of surface characteristics role in the chemo mechanical preparation and their role in par with hand instruments, effect of sterilization process, impact of hypochlorite on the surface of polished nickel titanium instruments .

Gary S.P Cheug et al(2007)[10]evaluated the LCF behavior of electro-polished and non electro-polished instruments in hypochlorite. Study design included 45 electro-polished instruments and 62 non electro-polished instruments. No of revolution, crack initiation sites and extend of crack propagation were noted. He concluded that no electro-polished instrument showed more than one crack. surface smoothness is enhanced by electro-polishing but did not protect the instrument from LCF failure.

Helio Perera lopes et al(2007)[14] evaluated the fracture resistance of NiTi SMA endodontic files. The helical plastic deformation and fracture morphology were evaluated by SEM. The results showed that there is significant difference in maximum fracture torque and there was a statistical deflection for the analyzed files.

Luis cha vez de paz et al(2007)[18]evaluated the presence of gram positive microorganism in endodontic instruments. Culture-based studies in Endodontics have more or less overlooked the significance of Gram-positive facultative bacteria in recent decades. By contrast, Gram-negative anaerobes have been extensively studied because of their frequent recovery in primary root canal infections and their association with acute manifestations of apical periodontitis.

Recent years have seen a renewed interest in Gram-positive facultatives as these organisms are common in samples from root-filled teeth affected by apical periodontitis. Structural components of the robust bacterial cell wall of Gram positives protect them from noxious environmental factors. Additionally, the majority of these organisms express fast-adaptive properties when exposed to extreme conditions, thus making them potentially interesting as causal elements in post-treatment endodontic disease. This review relates to different aspects of Gram-positive bacteria and their adaptive responses when being exposed to stressful conditions such as endodontic treatment procedures.

Damiano pasqualini (2008)[42]evaluated the effective shaping time and number of rotations required by rotary and hand Protaper in shaping simulated root canal. Group-1 specimen were shaped using hand Protaper and Group-2 specimen shaped with Protaper rotary. Number of rotation and effective time required were recorded and analyzed with non parametric Mann-Whitney U test. He concluded that rotary Protaper effectively shaped the simulated canal faster than hand Protaper.

Neechi et al(2008)[22] evaluated the mechanical behavior of the instruments by using Finite element analysis method to rotary endodontic instruments. Geometrical model of NiTi Protaper F1 instrument was created. The analysis of the thermo mechanical behavior of NiTi alloys was reproduced using an ad hoc test computational sub routine. He advocated that the instrument should be discarded after one use.

Vytaute Peciuliene et al(2008)[38] stated that a traditional concept is that apical periodontitis is the result of pathogenic effects of the microorganisms colonizing the root canal system and the response of the host defense system. The composition of the microflora of root canals differs in primary endodontic treatment and retreatment cases. Persistent disease in the periapical region after root canal treatment presents a more complex situation as it was thought earlier. Scientific evidence indicates that unsatisfactory outcome of cases in which treatment has followed the highest technical standards mainly is associated with microbial factors, comprising extra radicular and/or intra radicular infections

Gary S.PCheung et al(2005)[11]investigated the mode of failure of a NiTi instrument separation during clinical use. The study design included a total of 122 Protaper S1 that were discarded from a endodontic clinic in china. They were analyzed in SEM and classified the fracture to flexural and torsional. Out of 27 fractured instrument 2 files fell in the category of torsional fatigue and 27 files fell in the category of flexural fatigue. The mean length of the fractured segment in fatigue failure were significantly greater than that of shear failure. He concluded that macroscopic examination of separated instruments would fail to revel the true mechanism of failure and under high magnification is essential to reveal the feature that may indicate the possible origin of a crack and mode of failure.

Richard Gergi et al(2010)[27] evaluated the canal centering and comparison of canal transportation ability of Pathfile, Protaper and stainless steel files using computed tomography. The samples with severe curvature and short radius was selected . Canals were randomly divided into three groups and prepared with twisted file, Protaper and stainless steel files. He concluded that lesser transportation occurred in Twisted files rotary system. Highest transportation was observed in SS files and Protaper showed significant transportation when compared with Twisted files.

Iacoviello et al(2010)[15] stated work the stress-induced microstructural transitions and the crack initiation and growth mechanisms in a near equiatomic NiTi shape memory alloy have been analyzed, by XRD and SEM investigations. In particular, miniaturized dog-bone shaped specimens and a special testing machine have been used which allow in situ XRD and SEM investigations during mechanical loading, at fixed values of the applied deformation. Direct and reverse stress-induced phase transition mechanisms, between the parent austenitc phase and the product martensitic one, have been captured by X-Ray diffraction tests while the crack initiation and propagation have been observed by scanning electron microscopy. These analysis revealed that stress-induced transformations, from austenite to martensite, occurs near the crack tip, as a consequence of the highly localized stress, which significantly affects the crack propagation mechanisms with respect to common metals. In fact, blunting does not occurs during mechanical loading and, in addition, complete crack closure is observed during unloading, as a consequence of the reverse transformation from product to parent phase

Rui he et al (2010)[28] evaluated the influence of geometric features on the mechanical preparation of endodontic files using numerical stimulation. Finite element model of V taper instruments were developed and mechanical behavior of the file during bending and torsion was observed. He concluded that influence of flutes, helix angles have an impact over the mechanical properties such as bending in V taper files

Gambarini et al(2011)[9] evaluated the mechanical properties of NiTi instruments which are manufactured by different technique. Study included 40 K3 files and divided into two groups. One served as control. The second group of instruments (K4). All the groups were subjected to thermal treatment, stiffness and cyclic fatigue test were performed and data were analyzed. He concluded that manufacturing technique of K4 prototype instruments have enhanced the mechanical property when compared to the materials which are manufactured by traditional grinding process.

Sergio herrero moraes et al(2011)[31] evaluated the cutting efficiency of Protaper,Mtwo and K3. The study were divide into 3 groups, GP1- Protaper, GP 2-Mtwo, GP-K3. The files were prepared in the mesial canals of mandibular molar and analysed by ANNOVA and TUKEY. Results revealed difference in all the three groups. He concluded that among the three groups Protaper showed great cutting efficiency.

Shahra mazimi et al(2011)[32]evaluated the cyclic fatigue resistance and fracture mode of Race and Protaper instruments. The files were rotated in 30 or 60 degrees within the 2 or 5mm radius. The rotation of failure is analysed in ANNOVA and independent sample ‘t’test. Both the files exhibited more resistance to fracture and Protaper demonstrated highest number of cyclic fatigue and he concluded that radius of curvature was the main factor in torsional and flexural fatigue.

Andrea yamazaki arsaki et el (2012)[1] made a comparative evaluation of surface topography in the four different rotary system. They were divided in 4 groups. GP-1 K3, GP-2 Protaper universal, GP-3 twisted files, GP-4biorace. The instruments were autoclaved and observed in AFM and RMS values were tabulated. Result showed all the rotary files suffered surface wear with the change in topography in the active part of the file and he concluded that Protaper suffered greatest wear

Robertson et al(2012)[29] evaluated NiTi alloy metal fatigue resistance and fracture mechanism. He stated that Nitinol, a near equiatomic intermetallic of nickel and titanium, is the most widely known and used shape memory alloy. Owing to its capacity to undergo a thermal or stress induced martensitic phase transformation. Since its discovery in the 1960s, Nitinol has been used for its shape memory properties for couplings and actuators, although its contemporary use has been in for medical devices. For these applications, the stress induced transformation (‘super-elasticity’) has been used extensively for self-expanding implantable devices such as endovascular stents and vena cava filters, and for tools such as endodontic files. Most of these applications involve cyclically varying biomechanical stresses or strains that drive the need to fully understand the fatigue and fracture resistance of this alloy. Here we review the existing knowledge base on the fatigue of Nitinol, both in terms of their stress or strain life (total life) and damage tolerant (crack propagation) behaviour, together with their fracture toughness properties.

A.L Gloanec et al(2012)[12]evaluated fatigue crack initiation and propagation stages of a NiTi shape memory alloy are examined thanks to a low cycle fatigue interrupted test. Submitted to fatigue cyclic loading, the response of the alloy presents a classical pseudo elastic response. Two potential initiation crack areas are highlighted: at the phase interfaces or at the grain boundaries. Then, propagation results from the coalescence of many microscopic cracks. These two stages are detectable at the last 20% of the total fatigue life.

The study methodology comprises of 2 parts

1. Evaluation of surface topography of new and used Protaper , Mtwo, and wave one endodontic files using AFM
2. Ascertain the sterility of new endodontic files.

MATERIALS (AFM ANALYSIS)

1. Protaper NiTi rotary file (S1,S2,F1,F2).(Dentsply maillefer)
2. Mtwo NiTi rotary file (ISO size 10,15,20,25).(VDW GmbH)
3. Wave one primary file (size 25). (Dentsply maillefer)
4. 2.5% sodium hypochlorite.(Prime dental)
5. EDTA.(prime dental)
6. Saline.
7. Extracted mandibular molar.

ARMAMENTARIUM

1. ‘K’file ISO 10. (Dentsply Maillefer)
2. X-SMART DEVICE (Dentsply Maillefer).
3. WAVE ONE MOTOR(Dentsply Maillefer)
4. Glass slab.
5. Cyanoacrylate glue.(Anabond adhesive)

6. Ultrasonic cleaner.
7. Diamond disc.

SPECIAL EQUIPMENTS.

1. Atomic force microscope. (PARK SYSTEM)
2. Dental Lathe (Suguna dental lathe)

MATERIALS USED (MICROBIOLOGY)

1. Protaper NiTi rotary file (S1,S2,F1,F2). (Dentsply Maillefer)
2. Mtwo NiTi rotary file (ISO SIZE 15,20).(VDW GmbH)
3. Latex gloves.
4. Falcon tubes.
5. Cotton forceps.
6. Brain heart infusion broth.
7. Blood agar.
8. Incubator.

METHODOLOGY

Three NiTi rotary files system were used in the study.

They were divided into three main group along with three subgroups.

Group 1a -Unused Protaper S1,S2,F1,F2(.06 taper, 21mm)

Group 1b-Used Protaper S1,S2, F1,F2(.06 taper, 21mm)

Group 2a- Unused Mtwosize10,15,20, 25(.06 taper, 21mm)

Group 2b- Used Mtwo size 10,15,20,25 (.06 taper, 21mm)

Group 3a- Unused Wave one primary file (size 25,21mm)

Group 3b- Used Wave one primary file (size 25,21mm)

The NiTi instruments in group1,2 were used in the twelve mesial canal ofextracted mandibular first molar.Preparation were carried out by the operator using torque controlled reduction gear rotary hand piece (X-SMART-Dentsply Maillefer),according to manufacturer recommendation. The canal working length wasstandardized to19 mm. Canals werefilled with 2.5% sodium hypochlorite solution and the patency wasobtained using size 10‘K’ file(dentsply maillefer).Preparation was completed in the two groups as permanufacturer's instruction.

Group 3- The files were used in wave one motor with 6:1 reduction rotary hand piece (Dentsply maillefer) according to manufacturer instruction. Patency was obtained using size 10 'K' file(Dentsply maillefer). Canals were shaped using wave one primary file in pecking motion. Working length was checked when the instrument reached the middle third,and shaping was completed to the definitive working length. All the procedure was performed by the same operator.

PREPARATION OF THE SAMPLE

Before microscopic analysis the files used in the study were cleaned in a ultrasonic cleaner for 10 minutes, followed by cleaning in running water for 5 min and drying them using cotton cloth. Used and new files were arranged and positioned on the glass slab using cyanoacrylate glue.Each sample was placed in the platform to be viewed under AFM. Samples (files) were analyzed at 11 points along a 6 mm section from the tip of the file in needle mode operation. The scanning was carried out in room temperature and atmospheric pressure with 1 μ/s speed scan. Scanned areas were perfect squares (1μm×1μm).After analyzing the area, the values of the root mean square (RMS) were obtained. RMS or quadratic mean is a standard measurement of magnitude of a variable quantity in nanometers (nm). It evaluates the topography of the surface and the area in square micrometers.Three dimensional image is obtained and processed in XLE software.

PART- 2 (MICROBIOLOGY) METHODOLOGY

Two types of endodontic files were used in the study

New Protaper and M two are used.

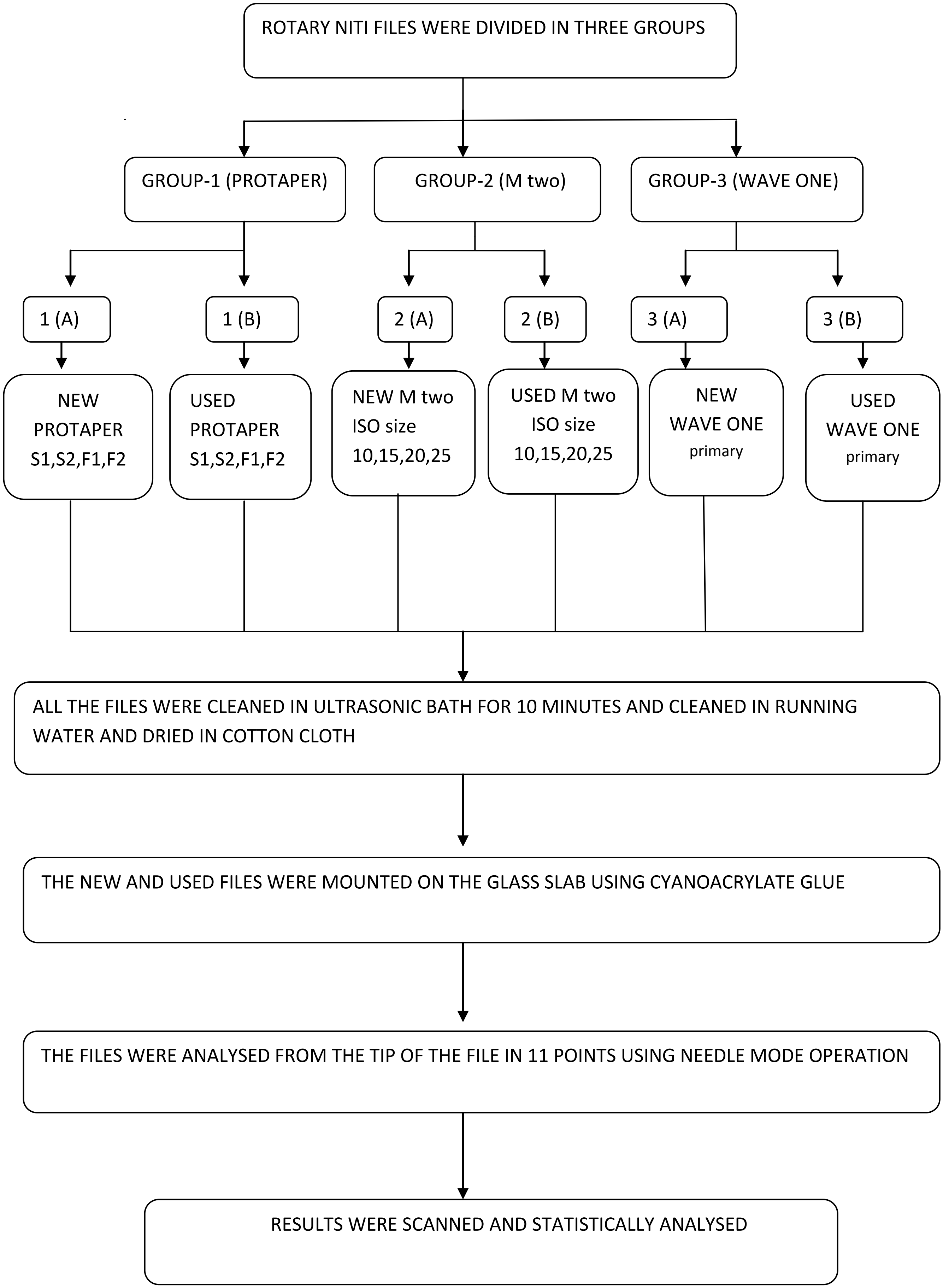
GROUP 1:

24 endodontic files (S1,S2,F1,F2)

GROUP 2:

12 endodontic files (ISO 10, ISO 15)

The new packs of endodontic files were opened using sterile latex gloves and then each file was transferred to a sterile Falcon tube containing 10 ml of sterilized Brain Heart Infusion (BHI) broth using sterile forceps. These test tubes without inoculation of the specimen served as negative control. All test tubes were incubated at 37°C for 24 hrs. Each tube was examined for turbidity. If the turbidity was not observed in 24 hrs those file are incubated for another 24 hrs.The tubes were photographed before and after the incubation period.Cultures from the turbid broth were inoculated on BHI agar plate and incubated for 24 hrs. The growth was observed and the colonies were identified.


ROTARY NITI FILES WERE DIVIDED IN THREE GROUPS
GROUP-1 (PROTAPER)
GROUP-2 (M two)
GROUP-3 (WAVE ONE)
1 (A)
1 (B)
2 (A)
2 (B)
3 (A)
3 (B)
NEW PROTAPER S1,S2,F1,F2
USED PROTAPER S1,S2,F1,F2
NEW M two ISO size 10,15,20,25
USED M two ISO size 10,15,20,25
NEW WAVE ONE primary
USED WAVE ONE primary
ALL THE FILES WERE CLEANED IN ULTRASONIC BATH FOR 10 MINUTES AND CLEANED IN RUNNING WATER AND DRIED IN COTTON CLOTH
THE NEW AND USED FILES WERE MOUNTED ON THE GLASS SLAB USING CYANOACRYLATE GLUE
THE FILES WERE ANALYSED FROM THE TIP OF THE FILE IN 11 POINTS USING NEEDLE MODE OPERATION
RESULTS WERE SCANNED AND STATISTICALLY ANALYSED

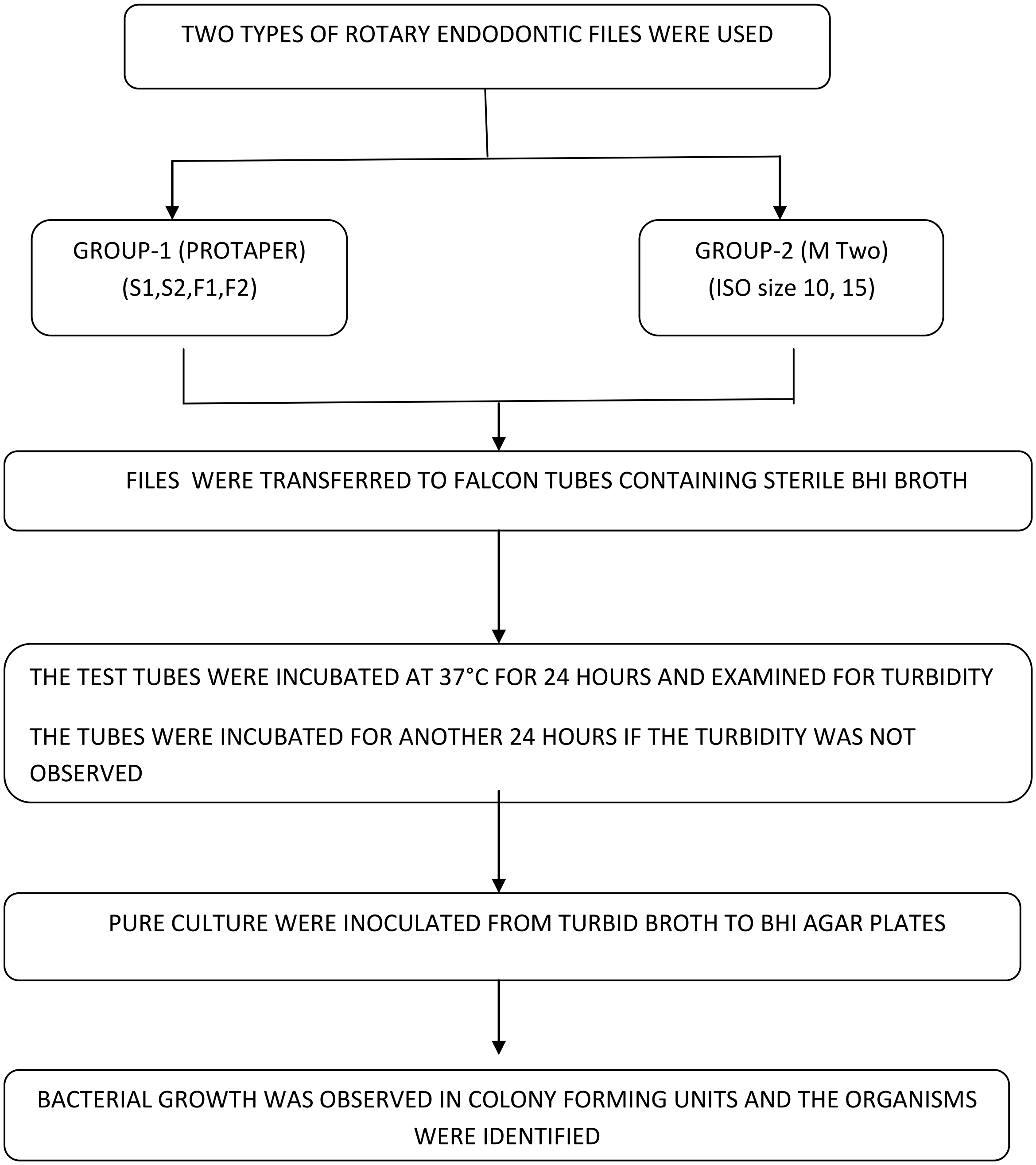

TWO TYPES OF ROTARY ENDODONTIC FILES WERE USED
GROUP-1 (PROTAPER)
(S1,S2,F1,F2)
GROUP-2 (M Two)
(ISO size 10, 15)
FILES WERE TRANSFERRED TO FALCON TUBES CONTAINING STERILE BHI BROTH
THE TEST TUBES WERE INCUBATED AT 37°C FOR 24 HOURS AND EXAMINED FOR TURBIDITY
THE TUBES WERE INCUBATED FOR ANOTHER 24 HOURS IF THE TURBIDITY WAS NOT OBSERVED
PURE CULTURE WERE INOCULATED FROM TURBID BROTH TO BHI AGAR PLATES
BACTERIAL GROWTH WAS OBSERVED IN COLONY FORMING UNITS AND THE ORGANISMS WERE IDENTIFIED

Fig-1 Clinical armamentarium

Fig-2 Wave one endodontic motor

Fig-3 Extracted mandibular molar

Fig-4 Dental lathe

Tooth were standardized to 19 mm by diamond disc

Fig-5 Protaper and mtwo used in

mandibular molar

wave one file used in mandibular molar

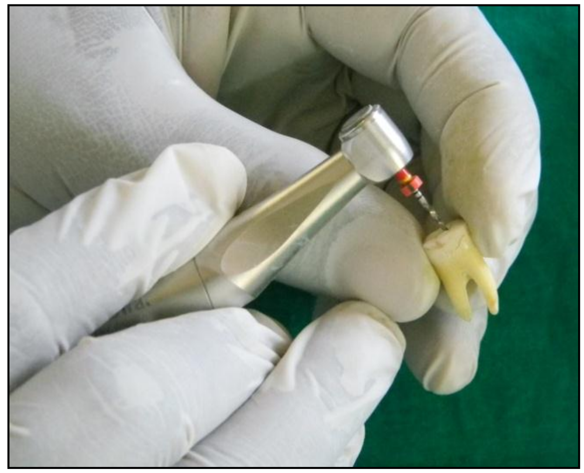


Fig-6 Samples were mounted in glass slab using cyanoacrylate glue

Fig7- Samples observed in atomic force microscope

7a

7a

Fig-8 Clinical armamentarium

Fig-9 Samples stored in incubator.

RESULT

Files in all the three group showed surface irregularities, and wear under experimental condition. The changes in the topography were recorded in the active working part of the file. Results were obtained in RMS (root mean square) for all the groups after analysis. Root mean square is a quadratic mean which is the statistical measurement of magnitude of variable quantity in nanometer. Statistical results showed there is a statistically significant difference at the level of 1% among the topography of the three groups.

Three dimensional image obtained from AFM reveals surface defects in new and used Protaper files. Using depth profile analysis (STM mode) the surface defects were quantified. It revealed the presence of micro crack measuring 0.732µm in new F1, pit measuring 0.977µm in new F2, and micro crack measuring 0.576µm in used F1 and pit of width 1.426µm in used F2, indicating the presence of pits and micro cracks in new and used files.

Table 1, 2, 3 shows the arithmetical value of RMS for the three experimental groups analysed by AFM. Finishing files showed greater wear than shaping files. Group 1(Protaper) exhibited more surface detoriation than group 3 (Wave one), and group 2(M two). Group 2 (M two) showed least wear among the three groups.

MICROBIOLOGY

36 files were randomly selected for evaluating the microbial contamination of the NiTi files. 2 files were discarded due to improper handling. Out of 34 inoculated samples 22 endodontic files produced turbidity after incubation in BHI broth for 72 hrs indicating that the files are contaminated. Incubation period was extended for an another 24 hrs to the samples which did not show turbidity. The sample tubes containing turbid specimen were selected for sub culturing to obtain a pure culture by re-streaking them in BHI agar plates. Microorganisms were observed by colony forming units. The organisms isolated were *Bacillus subtilis* and *Pseudomonas spp.*

PROTAPER

Image-1 New S1

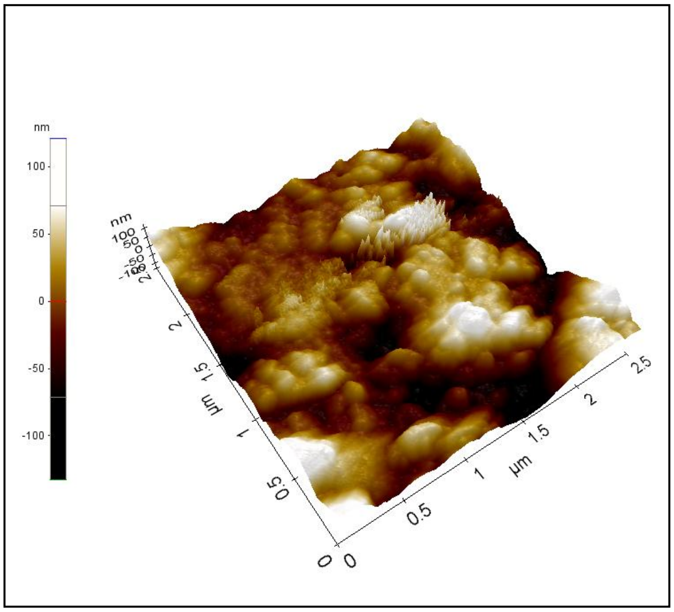


Image-2 New S2

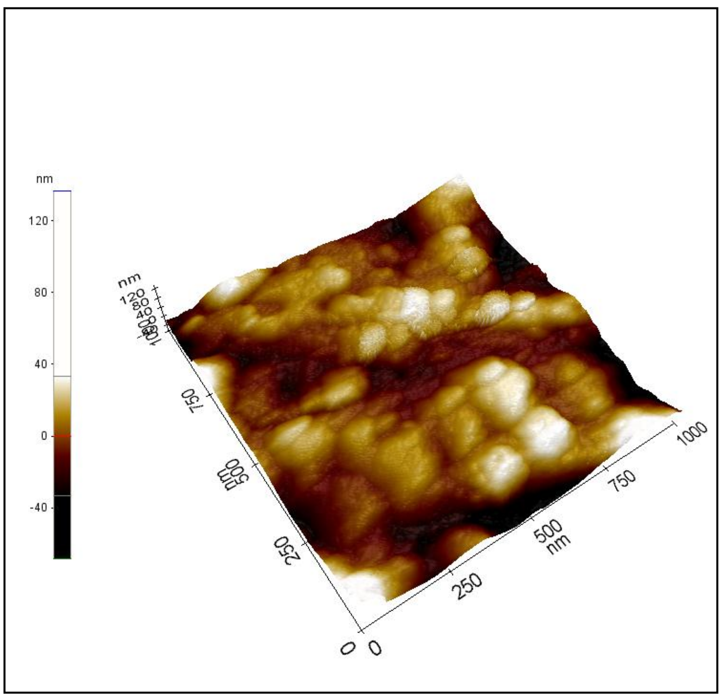
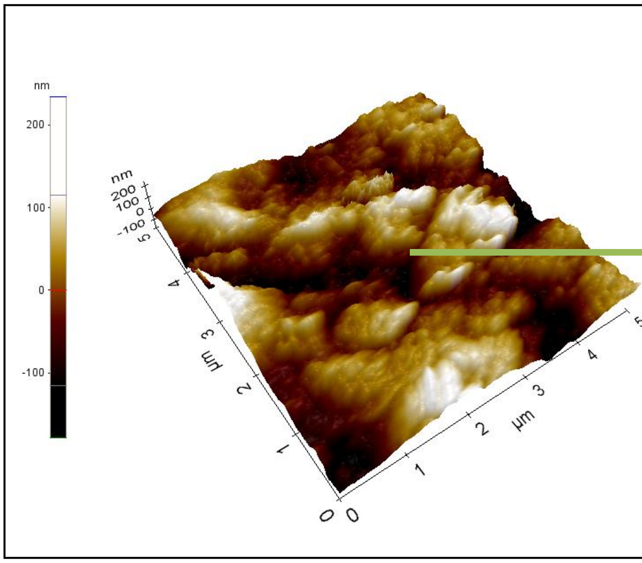


Image-3 <u>New F1</u>



SURFACE OF NEW F1 EXHIBITING A MICROCRACK

<u>DEPTH PROFILE ANALYSIS OF F1</u>

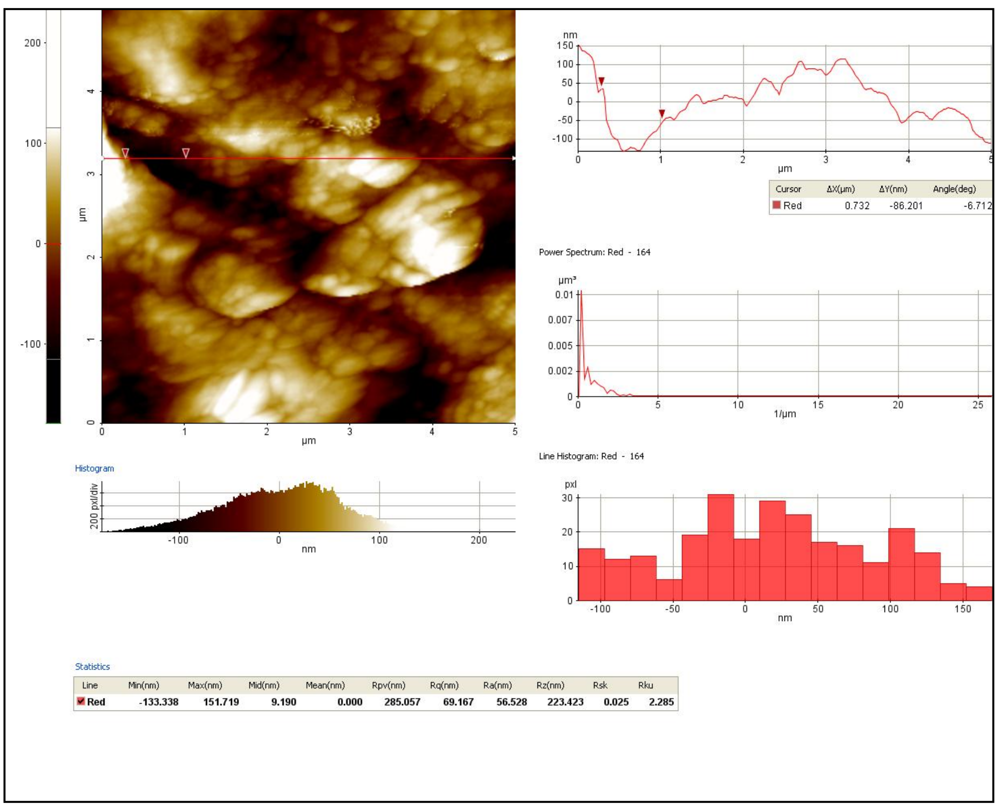
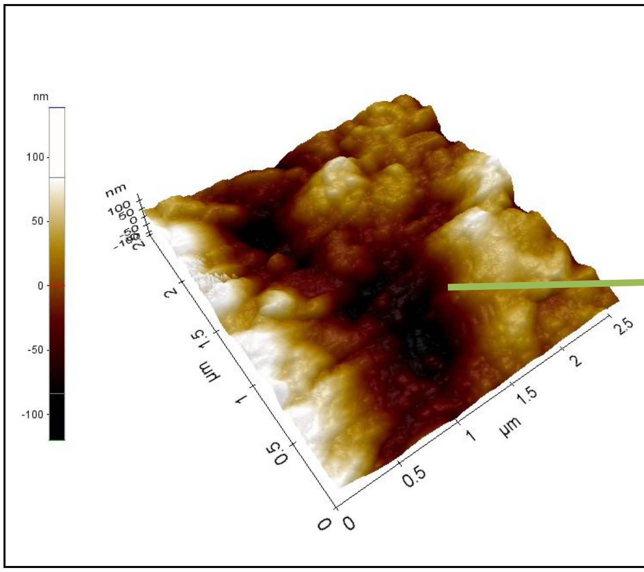
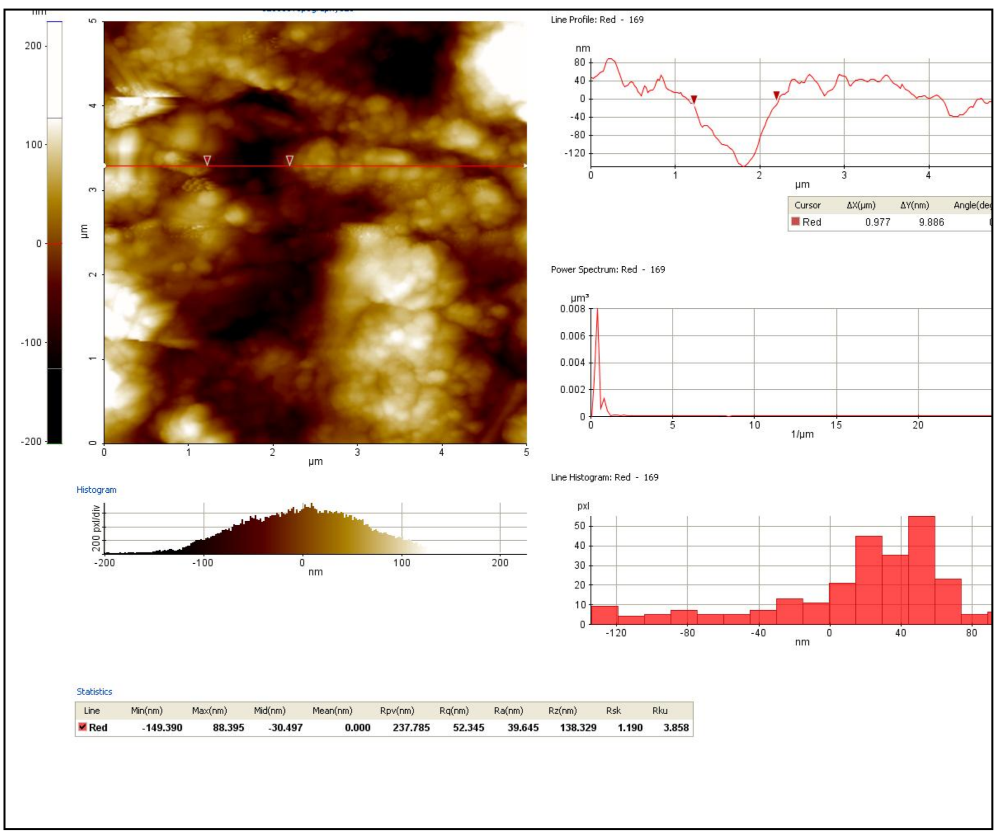


Image-4 New F2



SURFACE OF NEW F2 EXHIBITING A PIT

DEPTH PROFILE ANALYSIS OF F2



Line	Min(nm)	Max(nm)	Mid(nm)	Mean(nm)	Rpv(nm)	Rq(nm)	Ra(nm)	Rz(nm)	Rsk	Rku
Red	-149.390	88.395	-30.497	0.000	237.785	52.345	39.645	138.329	1.190	3.858

PROTAPER

Image-5 <u>UsedS1</u>

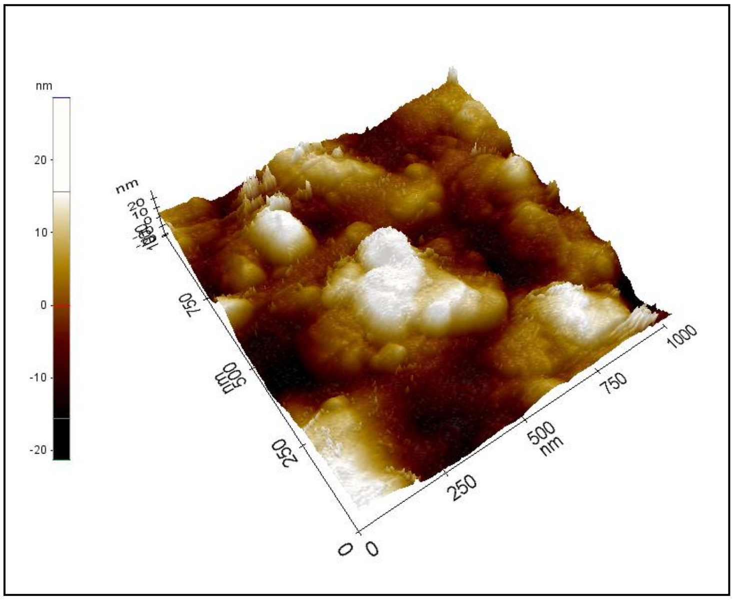


Image-6 <u>Used S2</u>

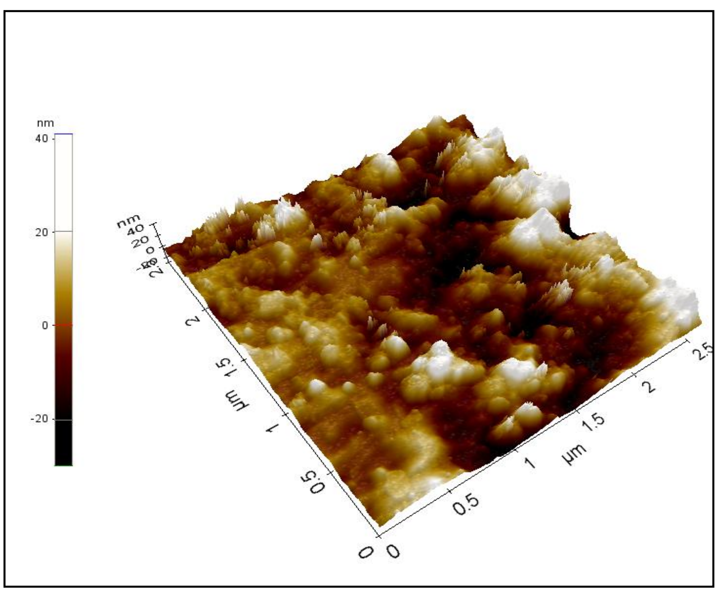
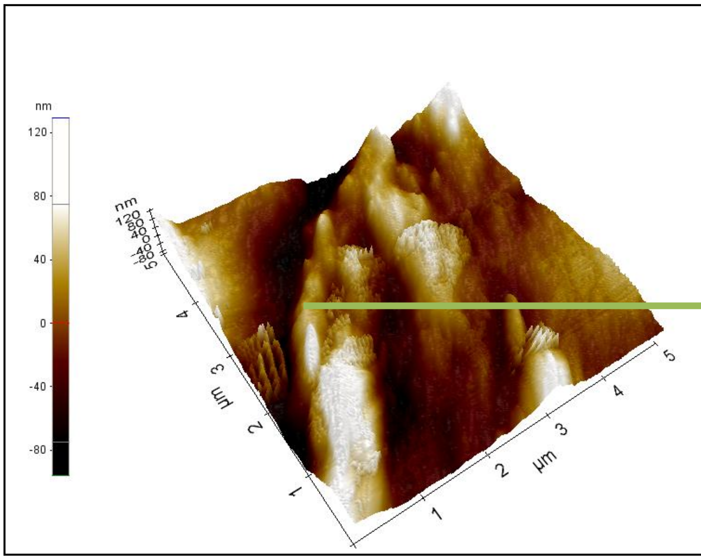


Image-7 Used F1



SURFACE OF USED F2 EXHIBITING A MICROCRACK

DEPTH PROFILE ANALYSIS OF F1

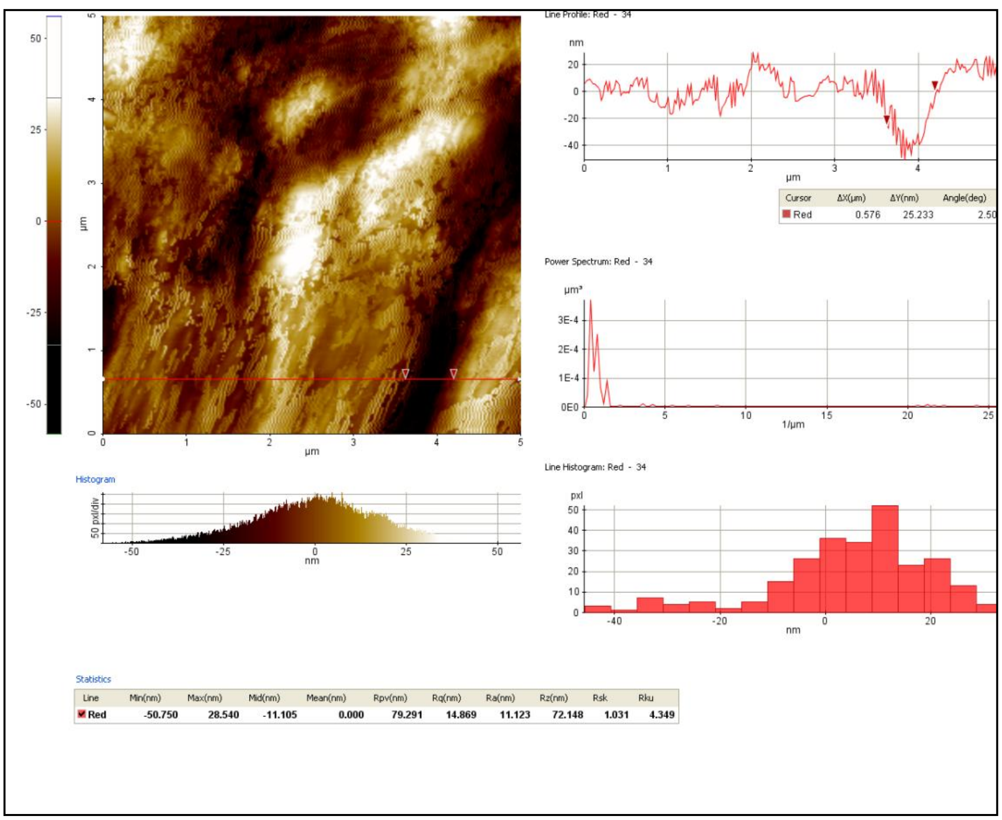
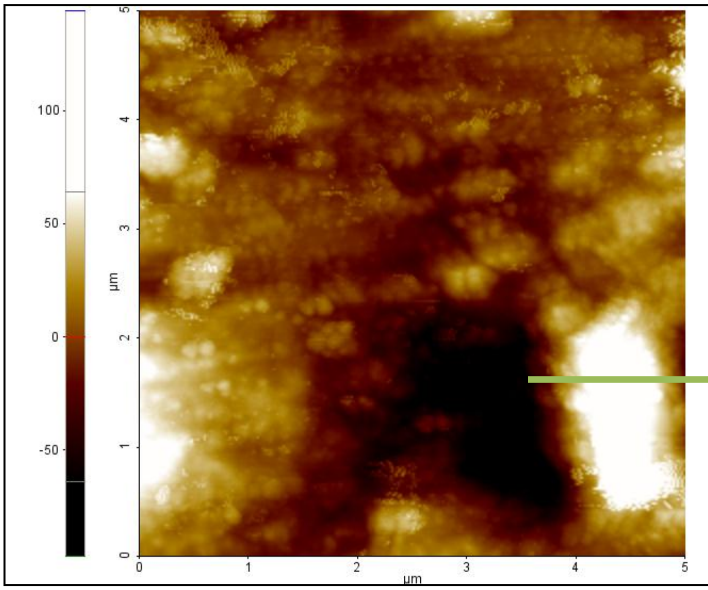
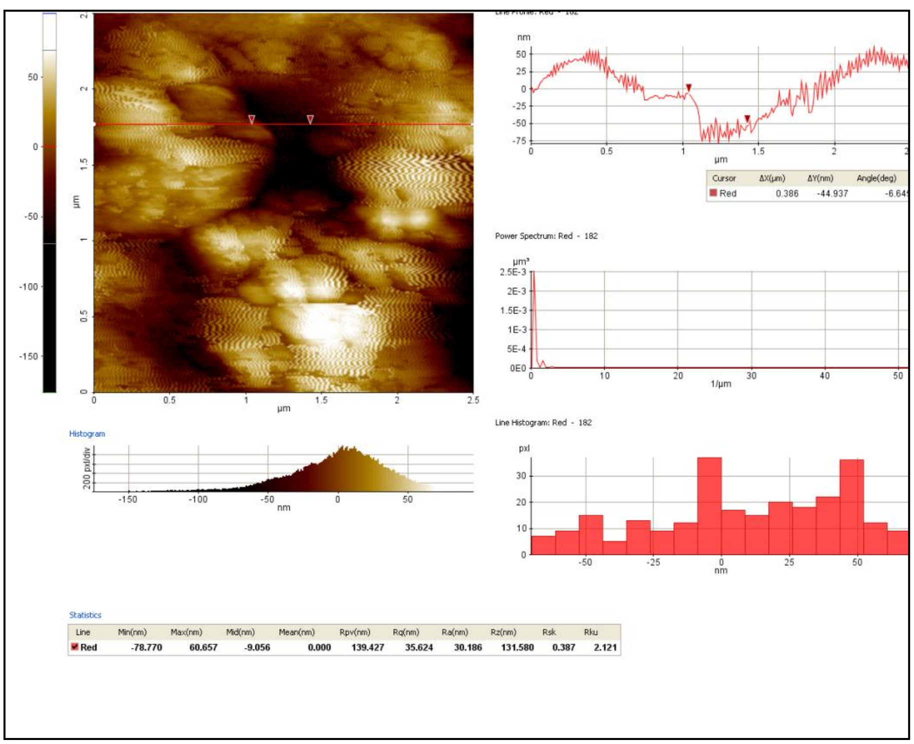


Image-8 <u>UsedF2</u>



SURFACE OF USED F2 EXHIBITING A PIT

<u>DEPTH PROFILE ANALYSIS OF F2</u>



M two NEW

Image-9 ISO size 10

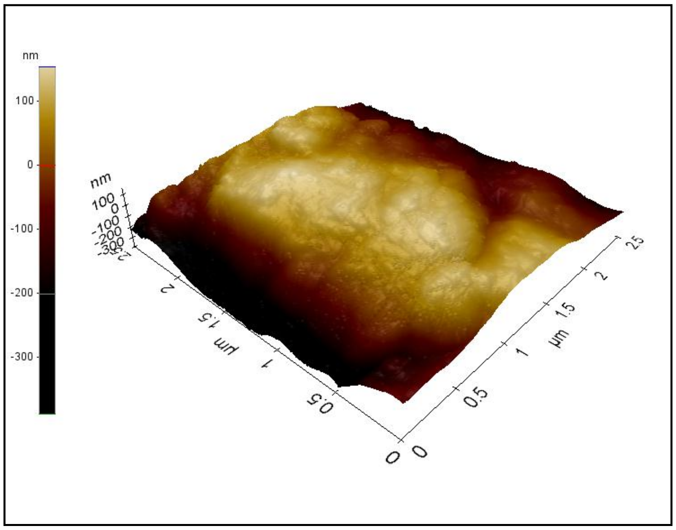


Image-10 ISO size 15

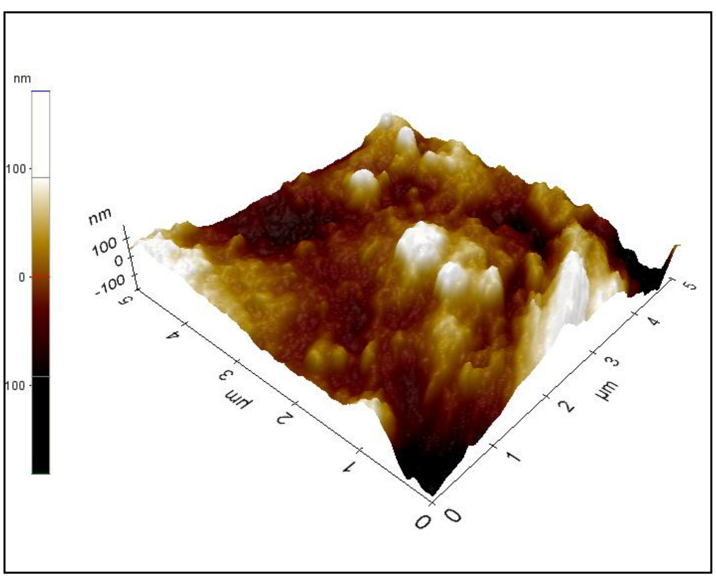


Image-11 ISO SIZE 20

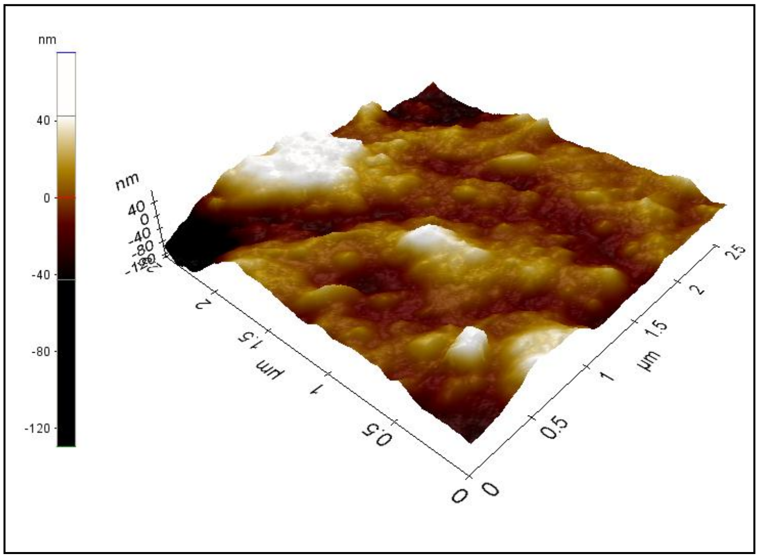
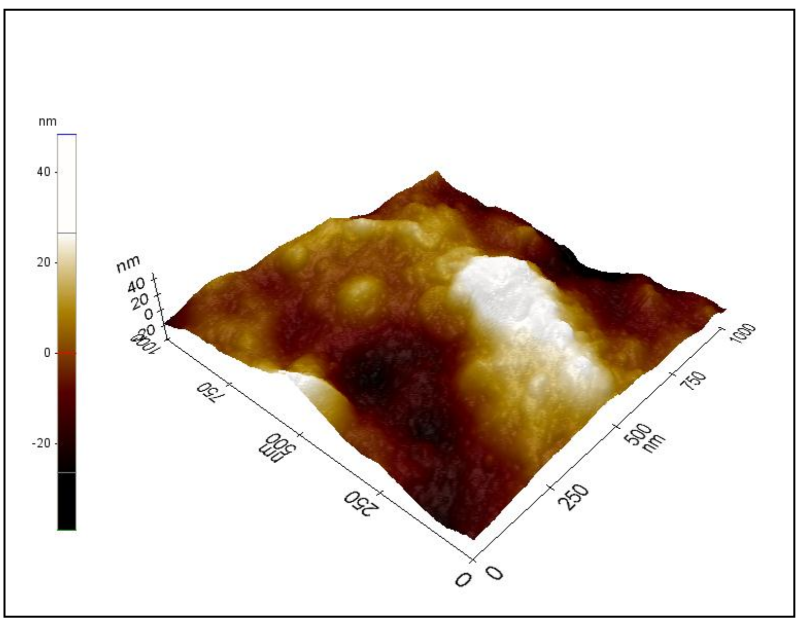


Image-12 ISO size 25



Mtwo used

Image-13 ISO size 10

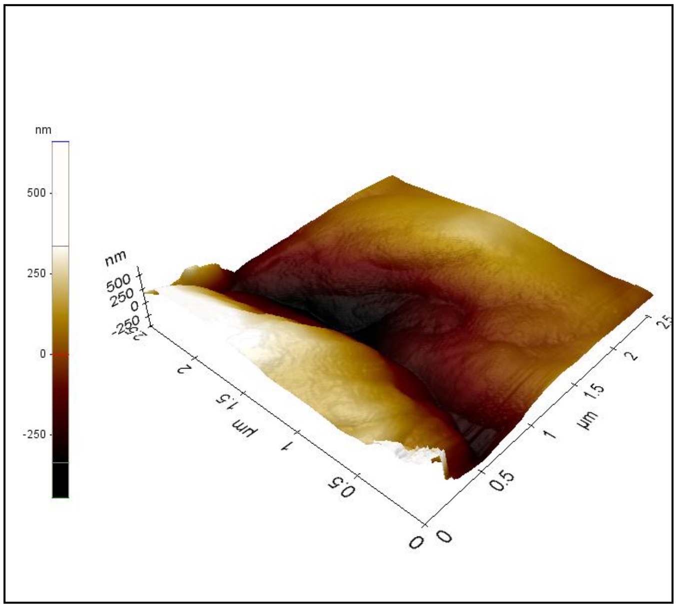


Image-14 ISO size 15

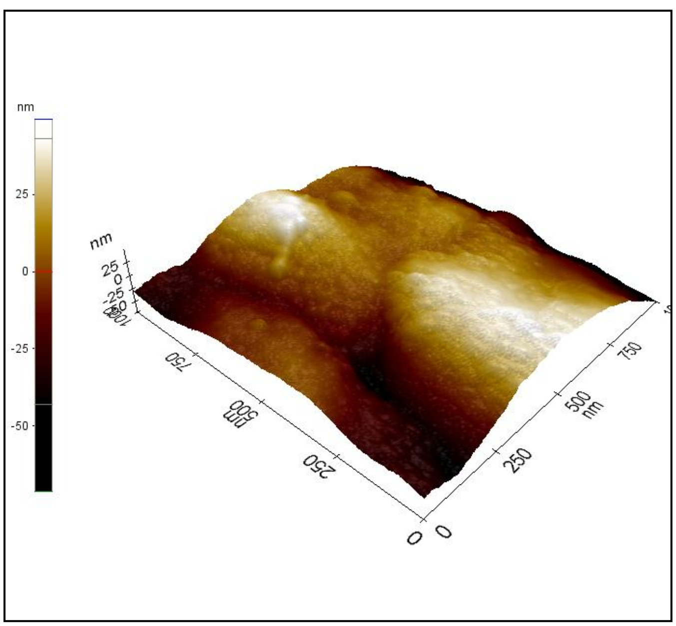


Image-15 <u>ISO size 20</u>

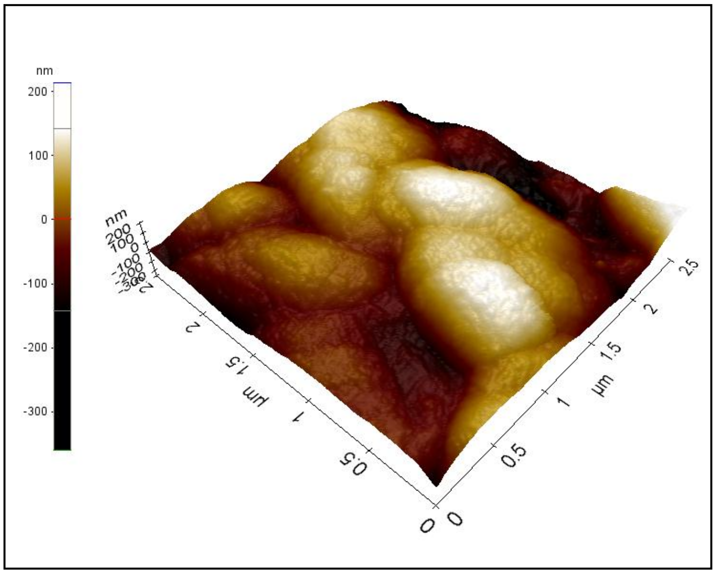
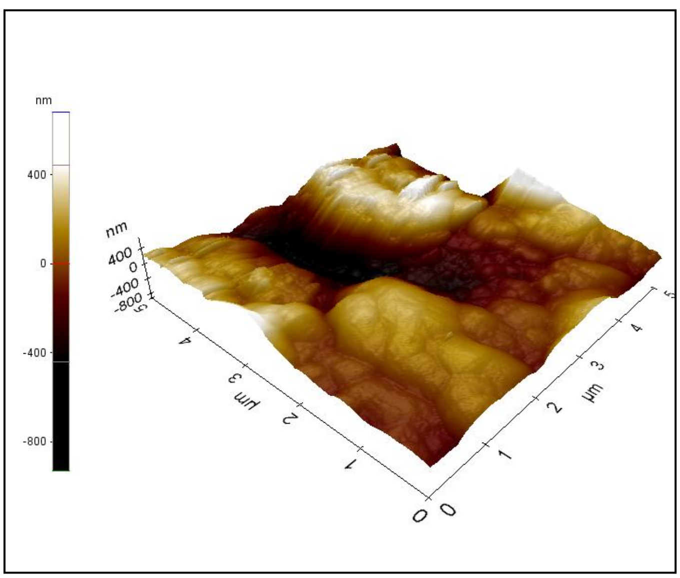


Image-16 <u>ISO size 25</u>



WAVE ONE

Image-17 WAVE ONE PRIMARY NEW

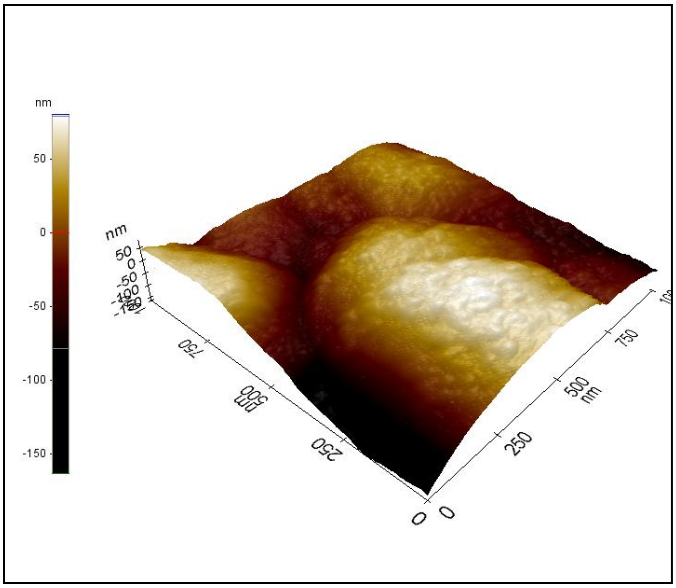


Image-18 WAVE ONE PRIMARY USED

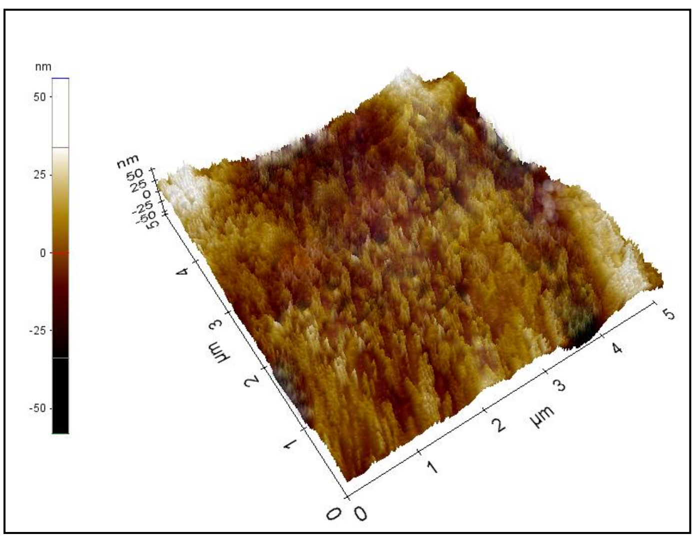


Image-19 <u>Group1 (Protaper)</u>

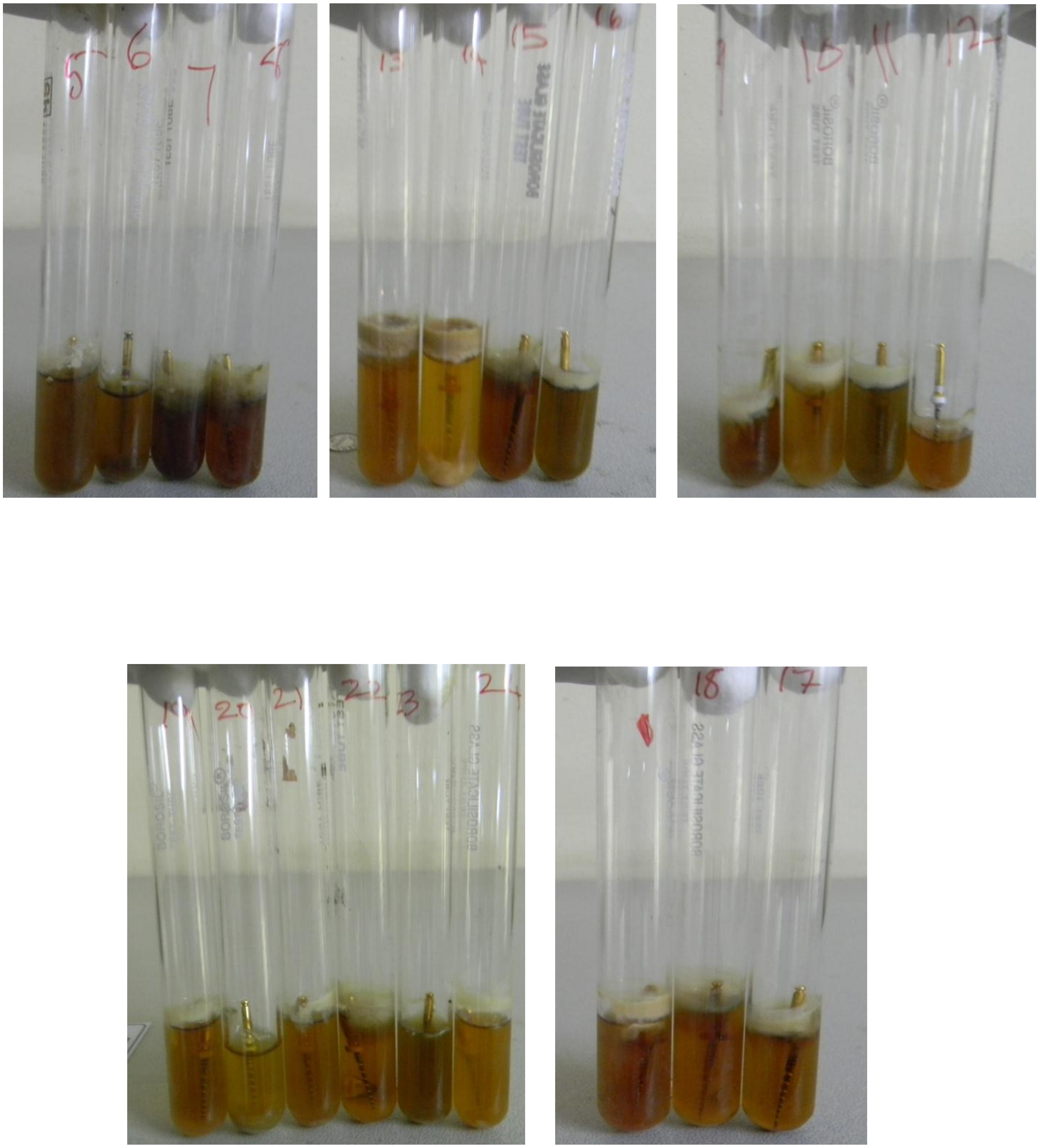


Image-20 Group 2 (M two)

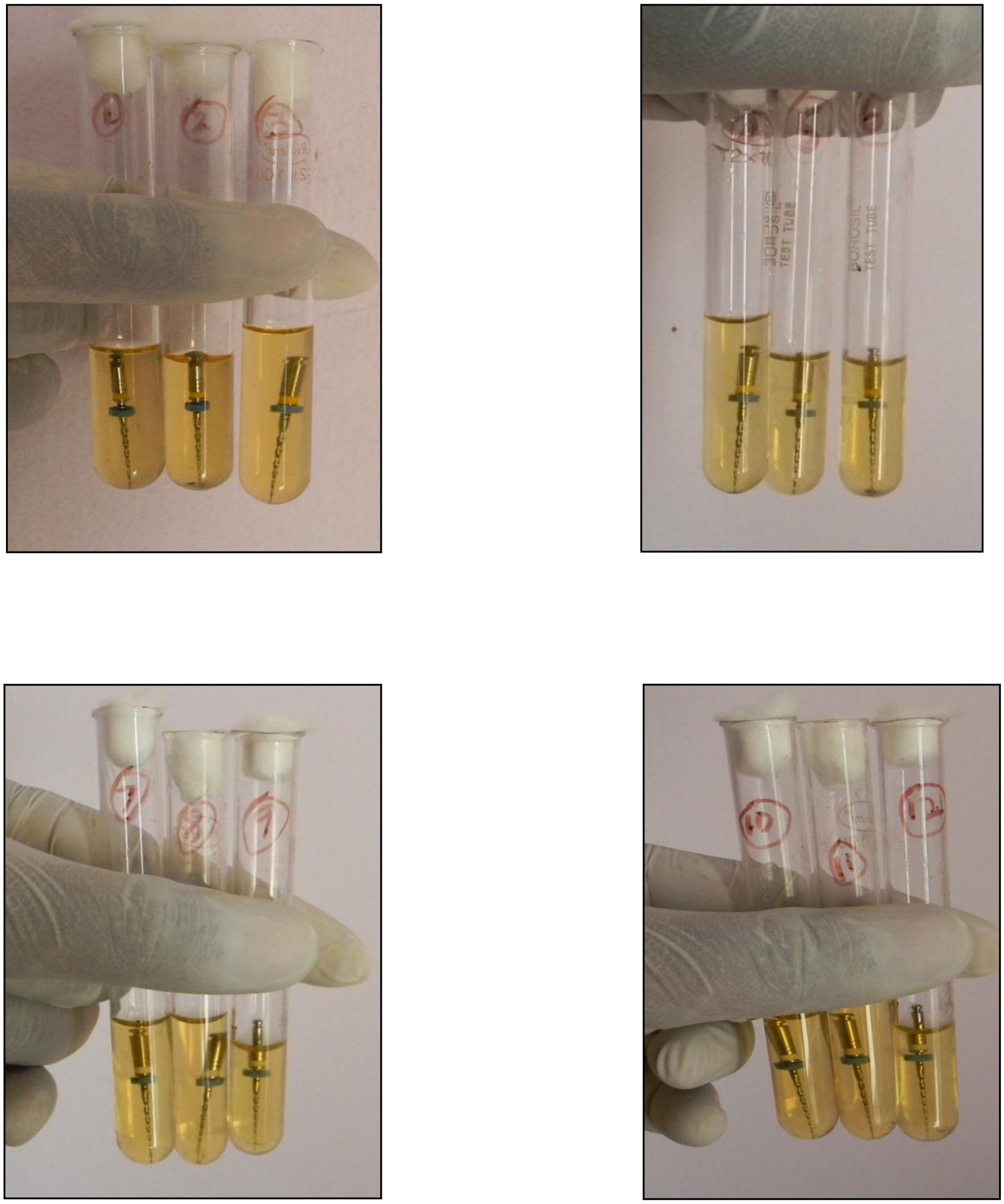
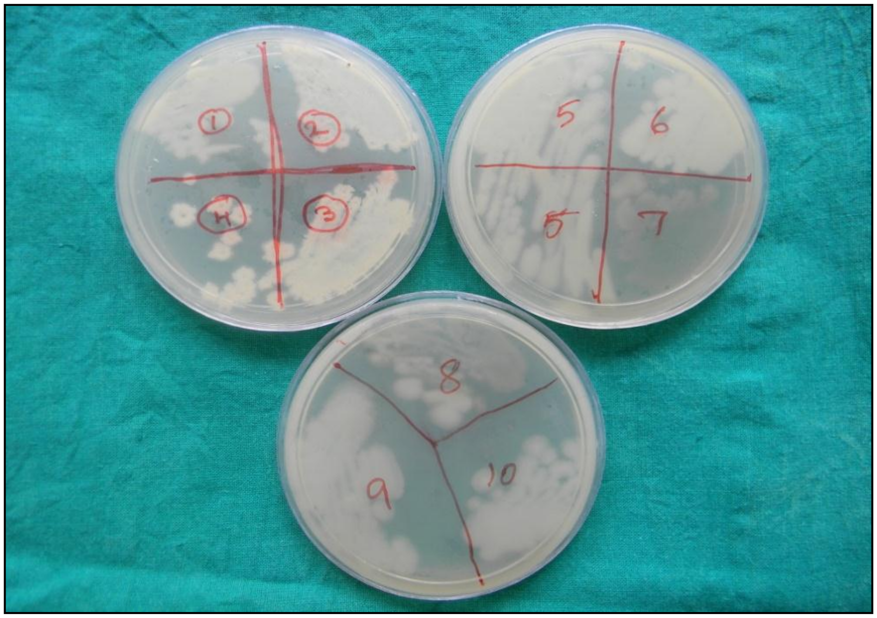


Image-21 G<u>rowth of microorganism in BHI agar</u>



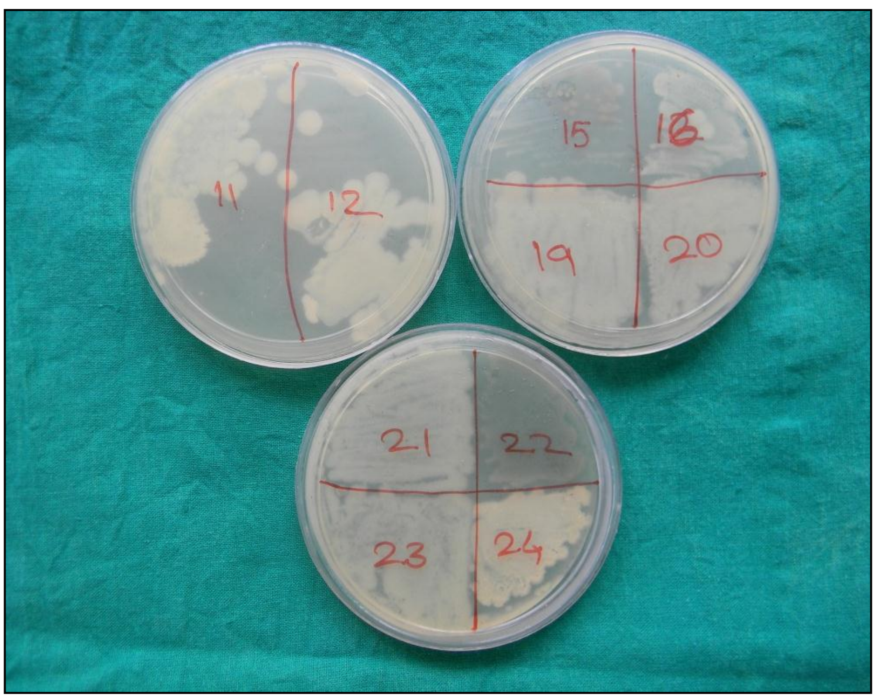


TABLE 1-RMS VALUE OF NEW AND USED PROTAPER

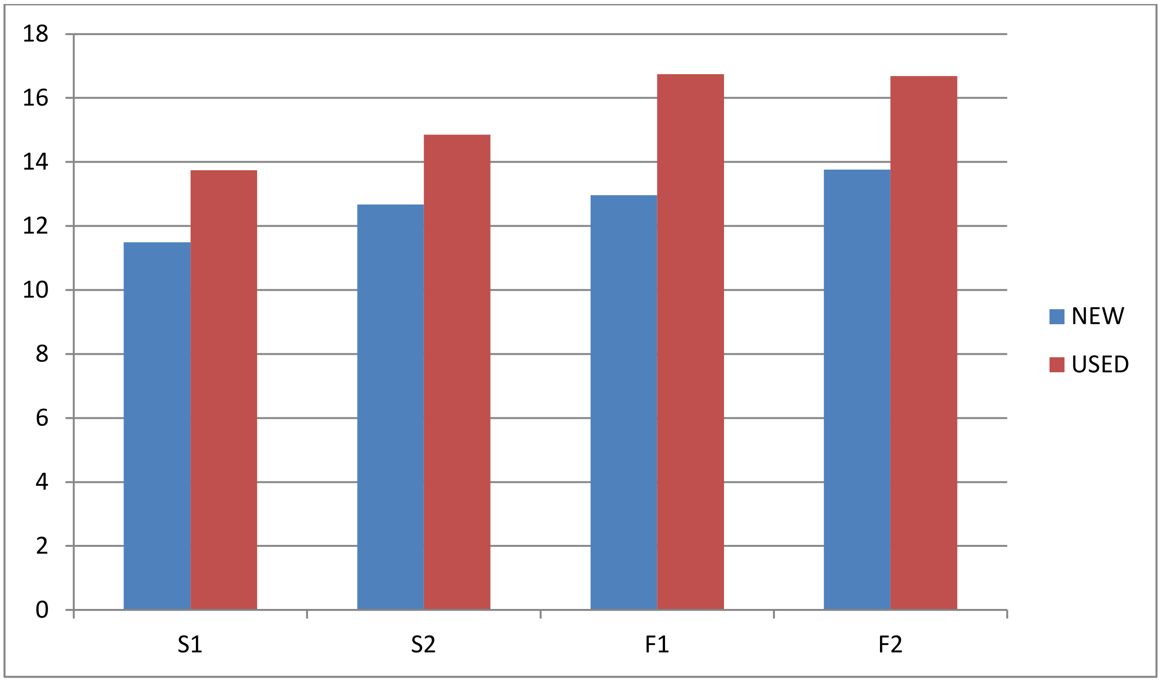


TABLE 2-RMS VALUE OF NEW AND USED Mtwo

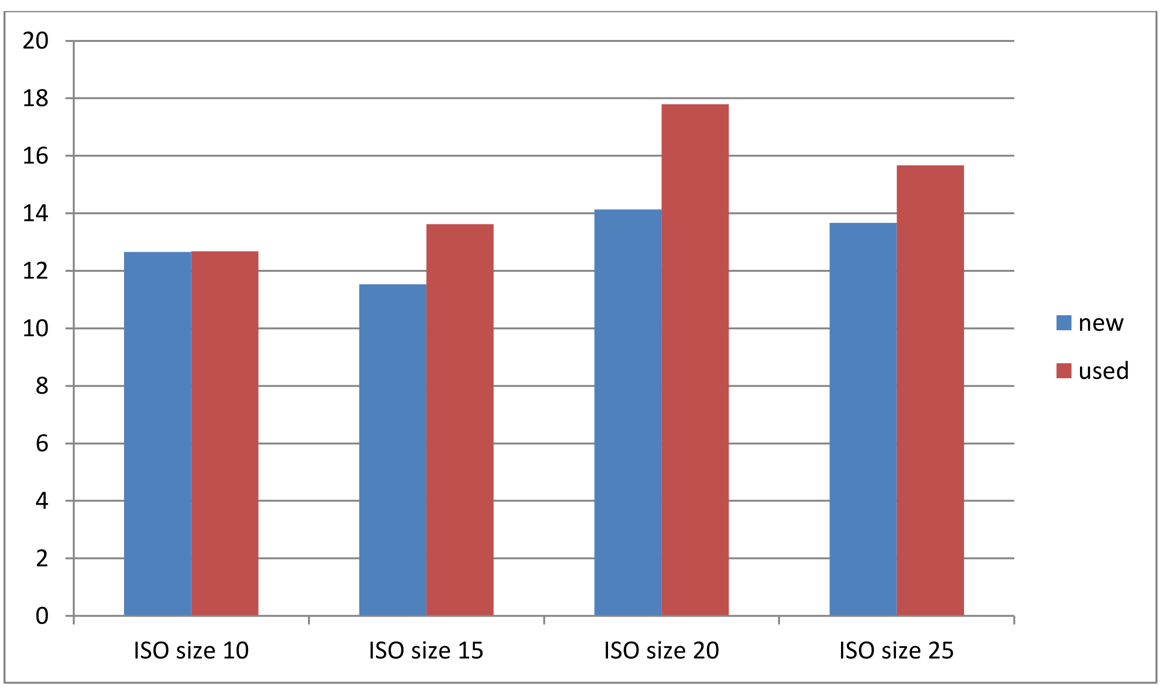


TABLE 3 -RMS VALUE OF NEW AND USED wave one

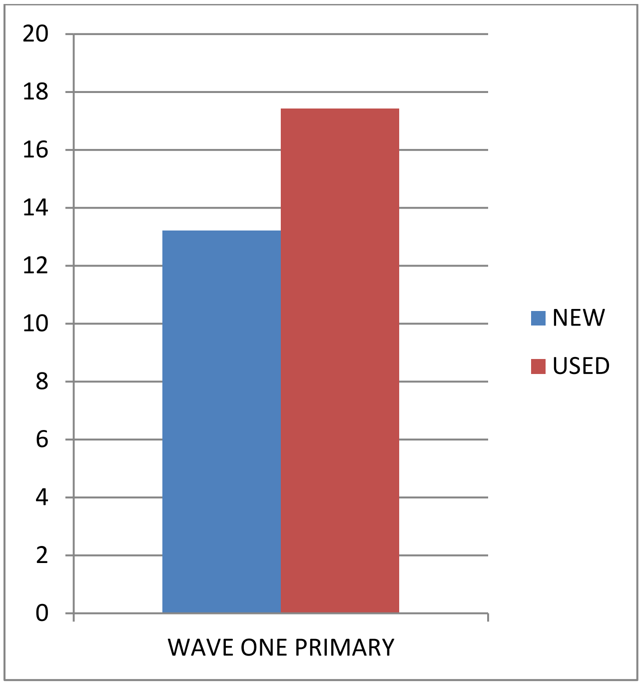


TABLE 4 – COMPARISION OF RMS VALUE FOR NEW FILES AMONG THE GROUPS

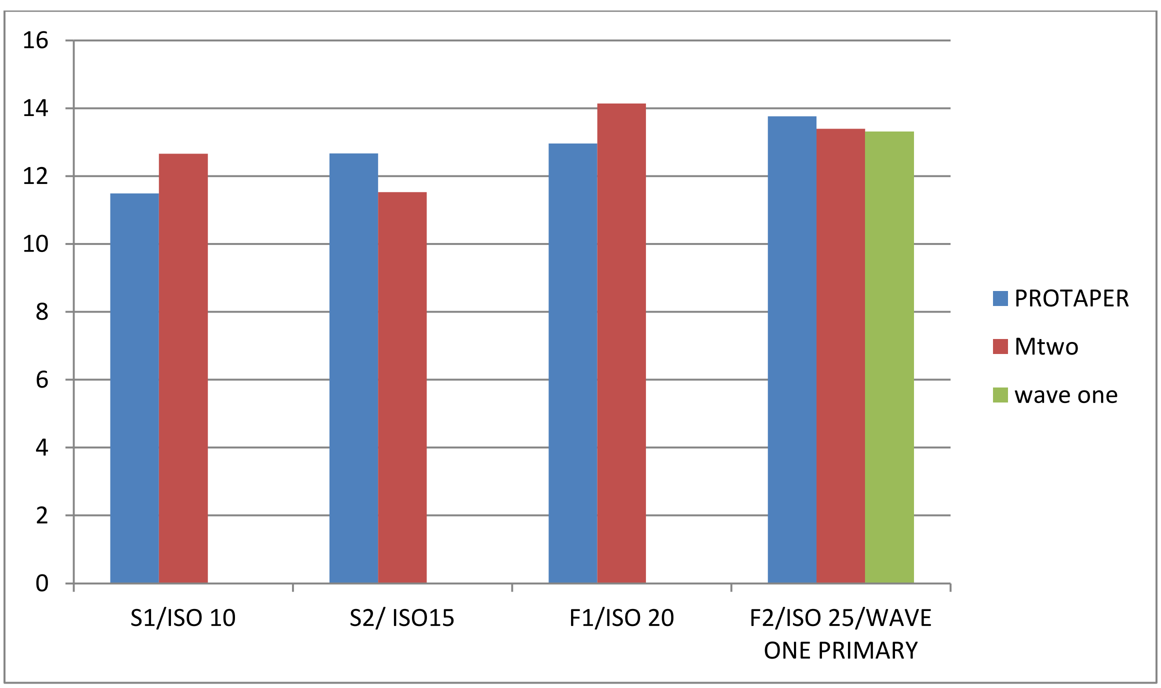
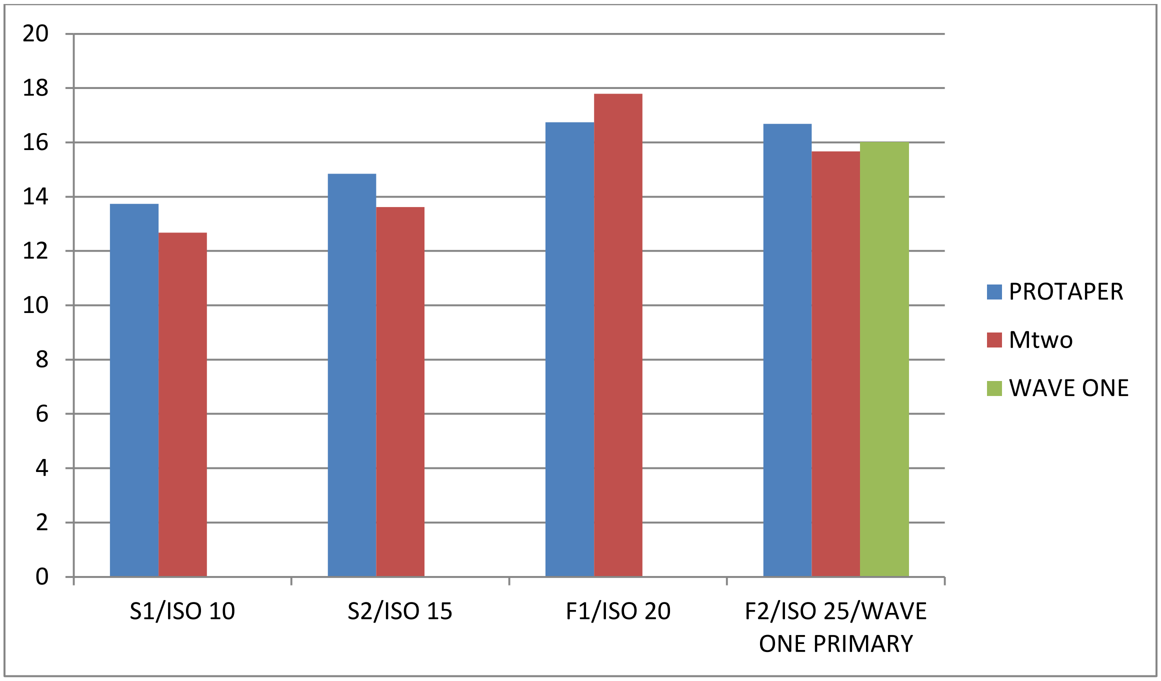


TABLE 5- COMPARISION OF RMS VALUE FOR USED FILES AMONG THE GROUP



DISCUSSION

The biologic objective of cleaning and shaping process is to remove all the pulp tissue, bacteria and endotoxins from the root canal system. Mechanical enlargement of the root canal system is therefore the most important part of the endodontic therapy. Further properly shaped canals are essential for adequate chemical irrigation and ultimately for proficient hydraulics required for a three dimensional obturation.[8]

Cleaning and shaping were initially carried out by stainless steel hand files which were used till late 90's. Stainless steel files are manufactured by twisting a square or triangular block of metal.[4] Inherent to, stainless steel files is that they have a high stiffness that increases with increasing instrument size and causes high lateral forces in curved canals. Along with the creation of an irregular cross-sectional shape the instrument rigidity of stainless steel files might leadto straightening of canals and transportation.[23]

The advent of NiTi rotary instrument have revolutionized root canal therapy by providing more predictable, centered and faster canal preparation than stainless steel files.[39]Nickel titanium alloy was developed 40 yrs ago by W.F.Buehler and Wang in naval ordnance laboratory in silver springs,Maryland, USA. NiTi NOL is acronym for the elements from which it has been formed Nickel, Titanium and Naval Ordanance Laboratory.[36]NiTi alloy used in endodontic instruments contain approximately 56%(wt) nickel and 44%(wt) titanium. Nitinol is called equiatomic since they containthe same amount of Ni and Ti atoms in one to one atomic ratio[4].NiTi alloy exhibit a greater strength, toughness and resiliency. It allows deformation up to 8% from which it is fully recoverable in comparision stainless steel which allows 1% recovery.[4] (Anderson et al, Morrow et al, Walia et al). NiTi alloy exhibit super elastic property, shape memory effect, and are corrosion resistant. Theabove mentioned properties are the main reason for the success of NiTi alloy in endodontics.[4]

Basically NiTi behaves like two different metals, as it may exist in one of two crystalline forms .The super elastic property (pseudoelasticity) of the alloy occur due to the change that results in transition of austentite to martensite due to the inherent ability of the alloy to alter its atomic bonding. The low temperature phase is called the martensitic or daughter phase(body centered cube lattice) and the high temperature phase is called as austentitic or parent phase(hexagonal lattice). Alteration in lattice occurs either dueto stress or change in temperature. During the root canal treatment a stress induced martensitic transformation occurs from austentitic to martensitic phase. Once the stress is relieved the alloy returns back to its initial lattice. This property of NiTi alloy have shown to provide more predictable root canal therapy without transportation. Inspite of all of their undeniable advantages the potential risk of unexpected breakage of NiTi instrument in the canal is a potential limitation which is faced by the operators.[4]

In general, fracture of metals can be classified as either brittle fracture or ductile fracture. In ductile fracture there is plastic deformation before it fractures. Material fails along the well defined crystallographic plane with the crack path travelling along grain boundaries. Brittle fractures are generally associated with little or no plastic deformation andoccurs in metals with poor ductility. Fracture occurs as soon as the cohesive strength of the material is exceeded. Crack front create ridges that spread along different plane within the alloy and radiates away from the crack producing the chevron pattern.(V-shaped markings pointing to the origin of the crack).[25]

As stated by Griffith law, once the fatigue strength of metal is reached NiTi alloy behaves as a brittle object. Once a crack is formed it is clear that the crack grows in length, and the stress required for propagation of the crack decreases.[10]Alpati et al observed the apparent widening of the machining groove and crack due to the accumulation of dentinal debris. Thus wedging action leads to the propagation of crack.[25]

Metal fatigue is the main reason for the fracture of rotary endodontic files.Metal fatigue occurs due to cumulative and irreversible changes within the metal due to repeated application of stress. It is also caused by tensile, compressive or shear forces as well as corrosion, wear changes due to thermal expansion or contraction.[25]

Metal fatigue of NiTi alloys can be classified into cyclic fatigue or torsional fatigue. Fracture because of torsion occurs when the tip or another partof the instrument binds in a canal whilst the shank continues to rotate. When the elastic limit of the metalis exceeded by the torque exerted by the hand-piece, fracture of the tip becomes inevitable (Martin et al). Torsional fatigue instruments shows deformation such as instrument unwinding, straightening, reverse winding and twisting.[30]Fracture because of flexural fatigue occurs when the instrument does not bind, but rotates freely in a curvature, generating tension/compression cycles at the point of maximum flexure until fracture occurs (Pruett et al,Haikel et al). Instruments which fracture due to flexural fatigue do not show any macroscopic evidence.[25]

Factors influencing instrument separation are instrument design, dynamics of instrument usage, canal configuration, instrumentation technique, number of uses, sterilization procedure, manufacturing process and manufacturing defects.[11]

Instruments resistance to fracture when it is subjected to torsional and flexural load mainly depends upon the cross sectional area and the file design. Instruments with large diameter and cross sectional diameter resist the torsional fatigue. These instruments resist torsional fatigue due to greater internal stress accumulation.[25]Berutti et al observed even distribution of stress in triangular cross sectional Protaper than in U fluted profile instrument. He also stated that instrument with small cross sectional design were more flexible but weaker when subjected to torsional stress.[6] Schafer et al later confirmed the result comparing five brands of Niti instruments and concluded that the file with largest cross section were the stiffest.[25]

Understanding the manufacturing complexity of NiTi NOL is the basic way to face the challenges regarding NiTi instruments.[4]Manufacturing of NiTi endodontic files is a complex procedure. It has to undergo a series of process such as wire drawing, degassing, fine wire drawing, annealing, profile drawing, cleaning and conditioning.[36]Unlike stainless steel files NiTi files cannot be manufactured by twisting because of it's super elastic and shape memory property. Therefore machining and grinding is the only way for manufacturing of NiTi alloys. Machining of NiTi wire is conducted at 220ft/min with carbide burs under active highly chlorinated cutting oil.Overheating of lubricating oil causes decomposition and oxidization which gets incorporated in the metal thus resulting in weak grain boundaries.[4]

Studies by Alpati and Flipi showed the occurrence of surface voids occur due to small amount of oxygen, nitrogen, hydrogen and carbon precipitates which get dissolved in the surface of the alloy during manufacturing.[25]It is possible that the machining of NiTi instruments may lead to irregular surface characterized by milling grooves, multiple cracks, metal flash, metal strips, region of metal roll over and pits (Alpati, Borgula, Marsicovetre, Eggert). Instruments of greater taper may exhibit more surface irregularity due to its more complex machining process.[25]Machining grooves play a major role in the initiation of microcrack.[11]The potential initiation of a crack site is either in phase interface or at the grain boundries (A.L.Gloanec).[12] Crack propagation is observed when there is stress induced transformation from austenite to martensite phase which occurs near the crack tip (F. Iacoviello).[15]

X-ray diffraction analysis and differential scanning calorimetry confirms that manufacturing process of NiTi instruments results in areas which are more prone to fracture. Regular transformation of alloy from austentic to martensitic phase can lead to changes in the mechanical property of instruments as well (Miyai).[25]

Surface finish has its influence in crack initiation process. Grooves which are left by the machining process may act as local stress risers which could initiate the crack progression. Once formed these cracks propagate to form a fatal catastrophic crack.[10]

In order to overcome these surface irregularities electro-polishing is done. Electro-polishing is a method of finishing the metallic product to high gloss. This method involves a controlled method for the finishing of the surface of the NiTi files. This process eliminates the surface defects and results in a smoother surface which is more resistant to fracture and corrosion. The process involves the alloy (acting as the anode) being submerged into an electrolytic solution (usually a combination of acids) containing a negatively charged cathode. A low current is passed through the solution,causing selective removal of protruding surface defects for NiTi alloys at a rate of 2.1 to 3.5 m/min. Electro-polishing have shown to improve the ultimate strength of NiTi alloy and the fatigue life of the instrument.(Miao et al, Tripi et al). However Borgula et al observed metal roll over and surface defects are observed even after electro-polishing. S.P. Cheung and Keven S Herold stated that low cyclic fatigue life of NiTi instruments are not enhanced by electro-polishing. The other modification of NiTi files to overcome wear property is carried out by ion and boron implantation on the surface of NiTi instruments. But these implantation were not frequently used by the manufacturer due to it's cost.[10]

It is important to evaluate the topography of the metal surface of endodontic files and their influenceon resistance to wear.Several investigations were carried out to find out the topography by magnifying loupes (Daughtery et al),low power microscopes (Sattappan et al)Operating microscope (Dygsten et al), Scanning electron microscopy (Sattapan et al, Shen et al, Luzi et al), mathematical finite element model (Neechi et al) and Atomic force microscopy (Inan et al, Topuz et al,Valios et al).[2]

ATOMIC FORCE MICROSCOPY is a cutting edge discovery in the field of nanotechnology. AFM has proven to be useful and a versatile tool to study the nanostructure and interfaces. It is also a useful tool to investigate the dynamic process in metal surface. AFM has been used in endodontics to provide qualitative and quantitative information about the topography of a large variety of metal materials.(Inan et al, Topuz et al, Valios et al). AFM analyse variety of surface and pens it down to nonometric scale. AFM works in 4 modes (1)-constant force mode (2)- contact mode (3)- non contact (4)- tapping mode.[17]

In AFM, a small tip is coupled to a cantilever, to which a support is fixed. This tip works as a probe to scan the sample surface. Interactive forces between thetip and the sample occur (Van Der Waals stresses), generating information about the surface topography. The samples are viewed by optical microscope which is the integral part of AFM before placing the working tip on the sample. AFM provides a tridimensional image of the surface. For the analysis, a small volume of the sample is used, and no special treatment is necessary, which differs from SEM, in which solid nonconductive specimens must be coated with a layer of electrically conductive material. AFM offers data x, y, and z of samples and, therefore, characterizes the surface of the endodontic instrument with a numerical description of its topography (Sugimoto et al)[1]. The main objective was to investigate the nature of the manufacturing irregularities and defects and their influence during instrument usage in root canal procedure.

Three types of rotary endodontic files were selected for the study, Protaper, Mtwo, and Wave one. They were subdivided into two groups,new and used. All the procedures were carried out by the same operator. The files analyzed in Group1(Protaper) areS1,S2, F1,F2, Group-2(M two) ISO size10,15,20, 25. Group-3(Wave one)ISO size 25(primary).

ProTaper NiTi instruments (Dentsply Maillefer, Ballaigues, Switzerland) have been developed to facilitate instrumentation of difficult and severely curved canals. ProTaper instruments have varying percentage tapers over the length of cutting blades. Another feature of the ProTaper instruments relates to their convexity, triangular cross-section, which enhances the cutting action while decreasing the rotational friction between the blade of the file and dentin which results in decreased instrument fatigue and potential for breakage.[16]

Mtwo endodontic instruments (Sweden & Martina, Padova, Italy) are a new generation of Ni–Ti rotary instruments. The transverse section of the Mtwo is an italic ‘S’ with two blade-cutting surfaces resembling that of the S-file. The helical angle of this file is variable and it increases from the tip to the handle. The helical angle is greater for the larger sizes (fewer flutes for instrument length), and decreases for the smaller sizes (more flutes). The rake angle is negative, and the tip is noncutting.[24]

The Wave One Endodontic system from DENTSPLY Maillefer is designed to provide simplicity and efficiency to the root canal shaping procedure. Wave One NiTi files are driven by the Wave One Endodontic motor which shapes root canals using a reciprocating motion. This reciprocating motion means the file continuously changes its rotating direction during the shaping procedure with a large rotating angle in the cutting direction (for high efficiency) and a smaller angle in the reverse requiring only one Wave one nickel titanium instrument to shape a canal in most cases (either Small, Primary or Large size) Wave One files are manufactured using the advanced M-Wire thermal treatment process, which provides greater flexibility as well as increased strength.[9]

The area occupied by crack has never been quantified[10].Cheung et al reported cracks which would have originated in the core of the instrument and could propagate to the pheriphery.[11]Classic fatigue phenomenon states that cracks begins from the surface and propagate inwards (Ewalds et al , Wanhill et al, Schijive et al).[10]This is the first study to evaluate the crack growth region quantitatively.

NiTi alloy are polycrystalline in nature. When the instrument is subjected to a stress induced transformation the first grain which undergoes transformation is the one which is subjected to tensile stress(Robertson et al).[29] The adjoining grain boundaries have been proposed to be the possible crack initiation sites(Robertson et al).[29]Crack propagation occurs in two forms (Gloanec et al) (1) martensite-martensite , martensite –austenite interface (2) along the grain boundries.[12]Progressive propagation of crack leads to the reduction in net area, hence the load bearing capacity reduces in such a way that it fractures in the next load cycle.[11]

In this study Group 1(Protaper) exhibited characteristic micro cracks and pitting in the active working section. Files which exhibited microcracks are New F1- 0.732µm, Used F1-0.576µm and pits were observed in New F2-0.977µm, Used F2-1.426µm. The other groups did not exhibit any such surface defects like microcrack or pits as observed in group 1(Protaper). Surface irregularities such as metal flash, deep milling marks, beach striations which were reported by Alpati et al, Berutti et al were not observed in the study.

No principle have been stated regarding the area of steady crack growth to strain. On the other hand extension of crack from the point of origin and propagation into the material is stated in Griffith law. NiTinol fractures at super elastic austenite state. The propagation of crack depends upon the yield strength and fatigue resistance of individual grain size. These surface defects can act like stress raisers which can propagate and may lead to instrument fracture.

Table 1,2,3 shows the mean RMS value of the all experimental groups.Quantitative results showed all the three groups irrespective of the manufacturer, type of alloy, suffered wear when subjected to experimental condition during canal preparation. Statistical analysis reveals a significant difference to the level of 1% observed in all the three groups.

RMS values were higher in used files than that of new files. In our study group-1 (Protaper) showed greatest wear than group-2(Mtwo) and Group-3(wave one). Group-2 (Mtwo) exhibited least wear among the experimental group.

Wave one files are manufactured with M-Wire technology. It improves strength and resistance to cyclic fatigue nearly four times in comparison with other brands of rotary NiTi files.(Brantley WA Pettiette MT, Delano EO, Trope).Improvement in the property of NiTi alloy(Wave one) could be attributed to an increase in the proportion of martensite within the material as a result of the heat treatment. Improvements in the mechanical properties of thealloy could also be related to partial annihilation of lattice defects that occur when the alloy is thermally treated. When the material is subjected to deformation or stress by machining a high density of lattice defects is produced as dislocations. When the metal is heated upto 400ºc, a recrystallization process can take place, decreasing the density of lattice defects and internal stress produced by work hardening. This increases the flexibility and strength of the Wave one file which resists wear and fracture.[9]

Protaper system defies imagination by having reverse and multiple taper within one file (Baumann et al, Turpin et al). Sergio Herrero Moraes et al tested the cutting ability of Protaper and Mtwo and K3 and concluded that cutting efficiency was high with Protaper which can lead to increased wear property.[37]In Protaper system, both S1 and S2 instruments have an increasing taper over the whole working range.S1 has a taper from 2% from D1 to 11% at D14, and S2 has a 4% taper from D1 to 11.5% at D14. The F1 and F2 instruments have a 7% and 8% taper at the first 3mm respectively. This may be the reason for more wear of Protaper which is accordance in this study.

To summarize all the rotary files observed in the study showed surface irregularity in spite of electro-polishing. Surface defects such as pits and micro-crack were found in both new and used files in group-1 (Protaper). The RMS value of the finishing files showed more wear when compared with the shaping files in allthe experimental group. The results of our study was in accordance with the study carried out by Cheung et al and Inan et al.

MICROBIOLOGY

The primary objectives of root canal therapy is to eliminate or reduce microorganisms in the root canal.Poor access cavity design, missed canals, inadequate instrumentation, inter-appointment cross infection, improper temporary or permanent restorations andmore importantly inadequate aseptic control are the main factors that can lead to the failure of endodontic treatment. Aseptic control involves the use of sterile instruments and sterile operating field during the procedure. Only limited study have been carried out to evaluate the microbial contamination of new unused endodontic files. This situation warrants, the investigation of unused endodontic files.

In thisstudy we have evaluated the microbial contamination of two rotary endodontic files system. The study consist of two groups, Group1- Protaper (6 files from S1,S2,F1,F2). Group-2 MTwo(6 files from ISO size 15,20). 34 files were used in the study.

In this study approximately 60% of the endodontic files received from manufactureris biologically contaminated.Only one set of culture condition were used to isolate the biologic contamination. Anerobic culture was not undertaken. The intension of the study is not to determine the exact microbiological load on endodontic files but to establish a fact that most of the new files are being contaminated with viable microorganism.

The organisms which were cultured from the file received from the manufacturer were *Bacillus subtilis* and *Pseudomonas.*

Bacillus subtilis, known also as the hay bacillus or grass bacillus, is a Gram-positive,catalase-positive bacterium which is resistant to penicillin. *B. subtilis* is rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions.*B.* subtilis is only known to cause disease in severely immune-compromised patients[43].

Pseudomonas is a genus of Gram negative aerobic gamma proteo - bacteria, belonging to the family *P seudomonadaceae.* As opportunistic pathogens, Pseudomonas spp. often invades the host tissue and cause infection and bacteremia in immune - compromised hosts *P seudomonas* - aeruginosa is increasingly recognized as an emerging opportunisticpathogenof clinical relevance. The members of the genus demonstrate a great deal of metabolic diversity, and consequently are able to colonize a wide range of niches.[38] Walshhave isolated strains of pseudomonas from secondary endodontic infection cases.[38]

No literature have been reported regarding the use of contaminated files leading to endodontic treatment failure, but there are chances of increasing the bacterial bioburden during the treatment leading to improper asepsis control.

In summary we conclude that new unused endodontic files were contaminated with viable microorganism. It is mandatory to clean and sterilize files before clinical use and one should not refrain from sterilization of endodontic files. It is suggested that manufacturers clearly label the sterility status of endodontic files.

SUMMARY

The purpose of the study was to evaluate the microbial contamination and surface topography of used and unused rotary endodontic files.

Three rotary endodontic file system were selected for the study. They were divided into three groups. Group-1(Protaper). Group-2 (M two), Group-3(wave one). The groups were further divided into two subgroups, new and used files. All the procedure were carried out by the same operator in the mesial canals of intact human mandibular molar. All the files were ultrasonically cleaned, dried and mounted in a glass slab with cyanoacrylate glue and were analyzed in 11 points along the 6mm apical section of the file. The scanned area were perfect squares. Quadratic mean value (RMS) was obtained and statistically analyzed.

Two rotary endodontic file system were used to evaluate the microbial contamination. They were divided into two groups. Group-1 (Protaper), Group-2 (Mtwo). 24 files from group-1 (S1, S2, F1, F2), 12 Files from Group-2 (ISO size15, 20). All the files were transferred to sterile falcon tubes containing sterilized BHI broth and were incubated. Each tube was observed for turbidity for 24 hrs. The tubes were photographed before and after incubation period. Pure culture were obtained by inoculation from turbid cultures into BHI agar plate and microorganisms were identified.

CONCLUSION

Analyzing the results from this study, it can be concluded that

1- Protaper (Group-1) reveals surface defects like pitting and micro-cracks in new and used files in spite of electro-polishing.

2- Electro-polishing does not leave the surface free from irregularity. Surface irregularity was observed among all the groups tested.

3- Among the three groups Protaper showed maximum wear in working condition followed by Wave one and M two.

4- M two (Group-2) had the least wear in terms of RMS value.

5- Microbial contamination was observed in 60% of the unused new files.

6- All the unused new files must be sterilized before use.

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